AN ANALYSIS OF CNS TUMORS IN SQUASH PREPARATIONS WITH HISTOLOGICAL CORRELATION

DISSERTATION SUBMITTED FOR

MD (PATHOLOGY)

September 2006

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI – TAMILNADU

CERTIFICATE

This is to certify that this dissertation entitled **"An Analysis of CNS Tumors In Squash Preparations With Histological Correlation"** is the bonafide original work of **Dr.Teleflo,** in partial fulfillment of the requirement for **MD (Branch III) Pathology** examination of the Tamil Nadu Dr.MGR Medical University to be held in September 2006.

Dean
Govt. Stanley Medical College and Hospital
Chennai - 600 001.

Prof. A.Sundaram M.D.
Head of the Department
Department of Pathology
Govt. Stanley Medical College,
Chennai - 600 001.

DECLARATION

I, **Dr.TELEFLO,** solemnly declare that dissertation titled, **"An Analysis of CNS Tumors In Squash Preparations With Histological Correlation"** is the bonafide work done by me at Govt. Stanley Medical College and Hospital during 2003-2006 under the expert guidance and supervision of **Prof.A.Sundaram MD, Head of the Department,** Department of Pathology.

The dissertation is submitted to the **Tamil Nadu Dr. MGR Medical University** towards partial fulfillment of requirement for the award of **MD Degree (Branch III) in Pathology.**

Place : Chennai

Date : **Dr.TELEFLO**

ACKNOWLEDGEMENT

I take this opportunity to express my heartfelt gratitude to **Dr.A.Sundaram,MD., Professor and Head of the Department of Pathology**, Stanley Medical College, Chennai for his keen interest, constant encouragement, guidance and valuable suggestions throughout this study.

I am extremely thankful to **Dr. V. Rajalakshmi, MD., DCP., Professor of Pathology**, Stanley Medical College Chennai who has extended her unstinted encouragement and guidance throughout the period of study.

My sincere thanks to **Dr.V. R. Mrinalini, MD., Professor of Pathology**, Stanley Medical College, Chennai for the constant encouragement and guidance extended to me during the study.

My sincere thanks to **Dr. R. Geetha, MD., Professor of Pathology**, Stanley Medical College, Chennai for the constant encouragement and guidance extended to me during the study.

I am extremely thankful to **Dr. K. Deiveegan, MS., Mch., Professor and Head of the Department of Neurosurgery**, Stanley Medical College, Chennai for providing timely material and encouragement throughout the study.

I am extremely thankful to all the faculty members of Neurosurgery, Stanley Medical College, Chennai for the help extended throughout the study.

Last but not the least I am grateful to all the faculty members, my colleagues and the technical staff members of the Department of Pathology, Stanley Medical College for their constant support during the period of study.

CONTENTS

Introduction

Introduction

The Brain is the most vital organ in our body. Tumors of the brain have unique characteristics that set them apart from neoplastic processes elsewhere in the body. Even though they amount to less than 2% of all malignant neoplasms their increased incidence in recent years has created great interest in studying them

Intraoperative consultation is an important component in the surgical management of brain tumors. Critical decisions regarding treatment and extent of surgical aggression can sometimes depend on an appropriate intraoperative cytodiagnosis. At the operating table many a times the neurosurgeon wants to know if the tissue taken for biopsy is from a representative site. The pathologist has to bear all this in mind and must be able to arrive at a diagnosis while the patient is at the operating table. The procedure adopted to achieve all these parameters must be simple, easy to perform, rapid and accurate. Crush cytology could meet most of these requirements.

Dr. William Cone was an early proponent of this technique as were Dorothy Russell and Lucein Rubinstein. Small fragments of tissue are crushed between two slides and then pulled apart to produce two thin

well prepared smears. It takes less than 5 minutes to prepare and stain the smear.

In Neurosurgical practice a rapid pathological diagnosis of the space occupying lesion of the nervous system helps the surgeon to monitor and modify the approach at surgery. If the biopsy tissue reveals a benign and surgically treatable condition it may be vital to proceed for complete resection by craniotomy. Hence it is vital to obtain an accurate pathological diagnosis of the lesion while the patient is still on the operating table.

The capability of diagnosing a lesion from a small tissue within a brief period of time is difficult with other methods. In experienced hands the smear technique attains a high degree of accuracy. But errors do occur and in that case decision should always be made on the basis of H&E stained paraffin section. Hence histopathology is still the Gold Standard in diagnosis of CNS Tumors though squash preparation has its advantages in terms of rapidity and timely patient management. Interpretation of crush cytology is based on the appraisal of many regions of a smear and often on one or more smears from the same biopsy but occasionally diagnosis can be made from a single field which

may be representative. Thus tissue smears are not purely cytological preparations, some intrinsic architecture still remains. It is important to have a proper approach to the examination of smears.

The entire clinical history, radiographic and CT findings should be known to the neuropathologist. In case of difficulties he/she should not hesitate to discuss with the neurosurgeon. As in any branch of diagnostic histopathology or cytopathology it is only with experience, that one's diagnostic expertise can improve. It is fair to say that one can attain an accurate diagnosis in 80-90% of smears.

Crush cytology is a simple, safe and easily accessible procedure but still it can only be used as a diagnostic adjunct to histopathology which is the Gold Standard for diagnosing CNS lesions.

An Analysis of brain tumors received at Stanley Medical College, Pathology Department was done by squash preparation with histopathological correlation to evaluate the usefulness of this procedure.

Aims and Objectives

Aims and Objectives

To do cytodiagnosis of lesion of Central Nervous System on Squash preparation

To correlate cytodiagnosis with histological diagnosis.

To assess the value of squash cytodiagnosis in intraoperative treatment plan.

To find out the pitfalls and limitations of squash technique

To study the age and sex incidence of CNS lesions and to compare the incidence with other studies as available in the literature.

Review of Literature

Review of Literature

Epidemiology

Brain tumors form less than 2% of all malignant neoplasms. The annual incidence of brain tumor ranges from 10-17 per 1,00,000 person. About 10% of primary brain tumors occur in the pediatric population. (Cotran et al 2004)

Age

Age patterns vary considerably depending on tumor type. Glioblastoma and Astrocytoma peak in the 65 – 70 year old. Incidence of Meningioma continues to increase as adult age increases. The peak age for Ependymoma and Medulloblastoma is in the 0 – 4 year age group. The peak age for Pilocytic Astrocytoma is 5-9 year old group.

Sex

Overall male is to female ratio is 1:1-1.6 (Preston – Martin et al 1993). Exception to this is Meningioma where the male is to female ratio is 1:0.4-0.8 (Preston – Martin et al 1993).

Race

Mortality and incidence rates of primary malignant brain tumors have been consistently higher in whites than in blacks (Anngers & Paleologos 1994) except Meningiomas (Preston – Martin et al 1989). Another exception is pediatric Craniopharyngiomas where incidence is higher among blacks than whites (CBTRUS 1996).

Social Class

Incidence of brain tumor is higher in the upper social classes (Vaughan & Schommer 1991) except Meningioma where the incidence rate is more in the lower socio-economic group (Henderson & Yu 1983)

Regional Variation

Brain tumor rates have been reported to be higher among Europeans and Israelis and lower among Africans and Asians living in Israel (Cohen & Modan 1968).

International Variation in Incidence of Brain/Nervous System Tumors (Age 35-64 Years) For selected High and Low rate region[ab].

Registry	Males	Females
High Rate Region		
Sweden	16.9	16.9
Denmark	16.5	15.9
Norway	15.2	15.5
Israel	14.3	15.3
UK South Thames	13.4	10.1
Poland Warsaw	12.5	8.1
USA Bay Area Whites	11.8	7.5
Low Rate Regions		
Slovenia	6.6	4.6
China	6.4	6.4
USA Puertorico	6.4	3.3
Belarus	5.9	3.7
Hong Kong	5.1	4.5
Japan Osaka	3.4	2.4
India Bombay	3.3	2.4

[a] selected regions where those reporting more than 100 male case
[b] table compiled forum IARC data for 1983-87 (Parkin et al 1992)

Aetiology of Brain Tumors

1. ***Radiation*****:** 3 forms of radiation have been implicated with respect to the development of brain tumors. They are
    i. Ionizing radiation
    ii. Non-Ionizing radiation
    iii. Radio frequency radiation

2. ***Inherited and Genetic factor:*** Inherited predisposition is estimated to account for approximately 5% of childhood central nervous system tumors (Bondy et al 1991). A number of inherited diseases have been associated with the occurrence of specific brain tumors. For example Turcot documented the occurrence of Glioblastomas, Astrocytomas and Medulloblastomas in patients with inherited adenomatous polyps (Turcot, Dispres and St. Pierre 1959).

   Studies of blood groups suggests that a higher proportion of individuals with Astrocytomas or Gliomas have blood group A (Sowbhaya et al 1991). The AB blood group antigens have been hypothesized to play a role in the modulation of cell membrane function (Engelmann & Schumacher 1993) and this role affects transport through the blood brain barrier thus providing a potential mechanism for affecting brain tumor growth. Molecular studies have also identified numerous genetic markers for brain tumors.

3. Occupation:

   i. Farmers are at increased risk due to exposure to pesticides and fungicides.

ii. Health professionals: Pathologist, Hematologist, Anatomist, Dentist, Embalmers, Nurses, Dental Nurses, Veterinarians are at increased risk. Formaldehyde exposure is suspected to be the primary agent for the induction of brain cancer. (Rusell et al 1998)

4. ***Diet:*** In 1994 Bunin et al found an inverse association with the intake of fruits, vegetables and vitamin C which block the endogenous formation of nitrosamine.

5. ***Immunosupression:*** In 1988 Patchell RA estimated that approximately 2% of all patients with prolonged immunosupression developed primary central nervous system lymphoma.

6. ***Virus:*** Clinical studies have clearly shown the presence of simian virus 40 (SV40) and related virus in human brain tumor (Martin et al 1995)

History Of Crush Cytology

Crush preparations for the diagnosis of lesions of CNS was first advocated by Louise Eisenhardt and Harvey Cushing in 1930. They used supravital staining for the diagnosis of intracranial tumors. The main advantage of this method was that it was possible to see under

the microscope cells in their entirety i.e. in their living state. It was helpful in differentiating the various types of Gliomas. The disadvantage of this technique was that it did not allow the specimens to be retained for subsequent review.

The present technique of crush cytology was introduced by Russell et al in 1937. 1% Aqueous toludine blue was used for staining and the technique has gained widespread acceptance.

Eisenhardt and Cushing et al (1930) said that in Pituitary Adenomas by staining unfixed squashed preparation with neutral red, they could differentiate chromophilic from chromophobic cell types. This was made based on differences in clear structure, presence of multiple nuclei and coarse granulations of chromophilic cells.

Arthur A. Morris (1947) described a new staining method called Reid stain in the rapid histological diagnosis. This stain which he described was developed by Dr. William Reid and used at the Montreal Neurological Institute. This method was simple and comparable with Hematoxylin and Eosin stain. The tissue when kept in an ice box could be accurately stained and interpreted as long as three days later. This method was not designed to replace the diagnosis by the

well established differential staining methods but provided a presumptive diagnosis during the surgery. The fastness of the stain varied. The preparation were good for at least three years. The stain gave an excellent differentiation of nuclei, cytoplasm, collagen, glial fibres and intracytoplasmic granules.

Russell et al (1947) published an account of the wet film technique on normal brain tissue. By this technique, even cellular material, which was drawn by tapping cysts, could be studied. It was better than frozen section, because every fragment removed could be studied in detail.

Later Papo and Colombo(1959) explained the limitations of using smear preparations in the intraoperative diagnosis of endocranial tumors.

It was William H Mc. Menemey who in 1960 emphasised that crush preparations were of real value to the neurosurgeon. His new method was not very useful for grading tumors by Kernohans system and the crushed preparations showed only approximately 66% correct diagnosis in comparison with paraffin sections.

Jane J.A and Bertrand G. et al (1962) did a study on the cytological method for diagnosing tumors affecting the CNS. They summarized that while frozen sections of samples subjected to rapid fixation produce the best specimens, crush preparations are preferred wherever a well equipped laboratory is not available.

Jane and Yason (1969) used Eosin and methylene blue and claimed that it was not possible to differentiate Chromophobe from chromophil cell type in Pituitary Adenoma.

Marshall, Adams et al (1973) evaluated the histological accuracy of the smear technique. The 190 consecutive smear biopsies examined in the Neurological Sciences Institute Glasgow during 1971 were reviewed and compared with subsequent paraffin sections for the same biopsies. In 94% of cases correct diagnosis was obtained from the smear. Most of the errors stemmed from the failure to distinguish between different types of malignant tumors. It was emphasized that this technique was the most appropriate available for rapid diagnosis.

Lawrence. F. Marshall and Bryan Jenett (1973) studied 187 smears and parallel paraffin sections for one year. They found out that the method proved reliable although in a few cases, malignant tumors

like Astrocytoma and secondary carcinoma could not be distinguished from each other. Burr hole biopsy combined with the smear technique proved safe and many patients with inoperable malignant tumors were spared of craniotomy. According to study by Kinoshita, Fekuiet et al (1978) the smear method was not popular in Japan. Out of 1447 histologically verified tumors studied from 1958 to 1976, in only 718 cases a smear preparation was done using eosin and methylene blue. In over 90% of cases they obtained correct diagnosis from the smear.

Gonzales, Campora, Hayends and Weller (1978) made a comparative study of Glioma cells in smear preparations and tissue culture by scanning electron microscopy. Smear preparations from 15 Malignant Gliomas, 2 Metastatic carcinomas and from normal brain were examined by scanning electron microscopy. Tissue culture preparations were also studied. Cells of Gliomas were stellate shaped and could be distinguished from myelinated nerve and from fibrin by their thickness and arrangement. The relationship of glial processes to blood vessels within the tumor was well demonstrated in smears. Metastatic carcinoma cells lacked the processes seen in Glioma cells. The relationship of the surface morphology of Glioma

cells in smears to the known invasive nature of these tumors were discussed. Berkeley Adams et al (1978) have published a paper on the smear technique in the diagnosis of neurosurgical biopsies. In 92% of cases they obtained correct diagnosis from the smears.

Adams, Graham, Doyle et al (1981) published in the biopsy pathology series, “Brain Biopsy – The Smear Technique For Neuro Surgical Biopsies”. In this they gave an account of the diagnostic accuracy of smear technique, its advantages, limitations, and an atlas for the diagnosis of tumors, reactive changes infarction and inflammation. They felt that smears should be examined with a good clinical history and radiological findings.

Gandolfi Tedeschin et al (1983) used squash preparation instead of frozen section, for rapid intraoperative diagnosis of three Neurilemomas of the thoracic spinal cord. Here some characteristic features like elongated wavy nuclei, absence of cytoplasmic outlines and dense fibrillary background were seen. Nuclear palisades with verocay bodies were also seen. The same authors presented a case in which smears and touch imprints of a parietal tumor revealed two distinct and easily recognizable cellular population. The predominant

population had an astroglial nature. The other element was the presence of unusually large giant cells with highly malignant features. This allowed the correct diagnosis of a giant cell Glioblastoma at the time of surgery. They also supported the interpretation of an astroglial nature of this tumor. Gandolfi A (1983) investigated a pituitary tumor with suprasellar and extrasellar extension by squash technique. The dominant cell type was large oval cells with finely granular cytoplasm. The nuclei of these cells contained inclusions of uncertain nature. Cells with eosinophilic cytoplasm in nests were also present. Hyperchromatic naked nuclei with various shapes were immersed in a homogenous faintly staining ground substance. Mitotic figures were also present. Cytology of these combined cell types allowed the intraoperative diagnosis of rather pleomorphic pituitary adenoma.

Mahadevan, Radhakrishnan et al (1984) highlighted their experience on 330 cases by confirming the histological diagnosis of intracranial space occupying lesions as obtained by squash preparation. Cahill, Hidvegi et al (1985) stated that the tendency of malignant Astrocytomas to grow along blood vessels could be appreciated only in paraffin embedded sections and not in crush preparations.

Xihua Yue, Xia Miu et al (1987) did not agree with the former authors and have stated that in their experience with crush preparations in 10 cases of Astrocytomas they tended to remain close to the blood vessels resulting in a distinctive papillary pattern. This feature according to them was best preserved by making slightly thicker crush preparations.

Nguyen, Johnson et al (1988) have described the cytology of Meningiomas and Neurilemomas in crush preparation as a useful adjunct to frozen section diagnosis. They have also highlighted on various characteristics that can be used to differentiate Meningioma and Neurilemomas in crush preparation.

Jan. F. Silverman, Douglas et al (1989) have published the cytopathology of neoplasms of the central nervous systems in specimens obtained by the cavitron ultrasonic surgical aspirator. The cytomorphological features of 22 central nervous system tumors in cusa were evaluated and compared with the findings in biopsy or resection. The preparation yielded well preserved cells similar to that described in crush preparations.

Cappa Bianca P et al (1991) said when stereotactic brain biopsy for toxoplasma encephalitis was done, identifying the encysted forms was usually easy, but identifying the tachzoites were not. However tachzoites could be readily identified in paraffin sections. In difficult cases a colloidal gold method was recommended to stain the parasite.

Nguyen GK et al (1992) compared cytomorphology of Pituitary Adenomas and Oligodendrogliomas in intraoperative crush preparations. He said that pituitary adenomas were characterized by single and clustered tumor cells with monomorphic round or vesicular nuclei that were commonly denuded of cytoplasm. Oligodendroglioma on the other hand showed cells with pleomorphic nuclei and wispy cytoplasm arranged in clusters around circular and empty spaces. In 1994 Johnson RS et al had given his experience in cytology of Central Neurocytoma in intraoperative crush preparation. Kristt D.A. et al in 1996 said that crush preparation is a rapid and informative means to facilitate pathological evaluation of fresh tissue from neurosurgical biopsies. Distefano D et al in 1998 reaffirmed the accuracy of cytological and frozen section intraoperative diagnosis in

neuropathology and the relevance of diagnostic accuracy during both craniotomic and stereotactic biopsies.

In 2001 Kumar P.V. et al studied intraoperative crush preparation smears of 19 cases of Medulloblastoma. The smears of undifferentiated type of Medulloblastoma which has a poor prognosis revealed tumor cannibalism, cytoplasmic vacuoles, target inclusions and prominent multiple nucleoli.

Pai R.R. et al (2001) emphasized the importance of intraoperative crush cytology in a 32 year old man. The case was diagnosed as Hemangioblastoma or Cystic Astrocytoma on CT scan. But intraoperative crush preparation confirmed it to be a Choroid Cyst Papilloma. The utility of crush cytology in the rapid diagnosis of CNS tumors and the differential diagnosis of CNS papillary lesions were highlighted in this study.

Chen K et al (2005) reported on a case of Pituicytoma and discussed how the crush smear could be differentiated from Pituitary adenoma, Astrocytoma, Meningioma and Schwannoma. Danesh bod Y et al (2005) studied 72 cases of intraoperative crush preparations of Craniopharyngiomas and concluded that with good clinical history,

crush preparation cytology plays an important role in the diagnosis of central nervous system tumors.

Smear Histology

Normal cytological appearances of various areas of brain are discussed in great detail which would help us to differentiate various cell components in the smear. (Gray's et al 1980)

Normal Neuropil

Normal Neuropil will show characteristic and distinctive blue staining. This staining is uniform in normal brain.

Cerebral Cortex

Cerebral Cortex will show numerous neurons of varying sizes, glial cells and small blood vessels. The large neurons or Betz cells will be seen in the motor cortex.

Thalamus

Thalamus will show scattered neurons and glial cells.

White matter

White matter will show cells which vary greatly in size and shape. There would be numerous blood vessels of varying caliber. Capillaries will be particularly distinctive. Neurons will be absent.

Basal Nuclei

Basal Nuclei will show few but large and conspicuous nerve cells. The number of nerve cells would be less in comparison with cerebral cortex

In Hippocampus

In Hippocampus an admixture of large nerve cells and small hyperchromatic neurons from the fascia dentate will be seen. Large and small neurons would be recognized because of their shape, appearance of the nucleus, presence of Nissl substance and blood vessels. Microglia and Oligodendroglia would have a rather densely stained nuclei and nuclei of microglia will be elongated. The nuclei of astrocytes would appear clear and transparent.

In Cerebellum

Cerebellum will show numerous granule and purkinje cells

Choroid Plexus

Choroid Plexus will show hyperchromatic cells arranged in papillary pattern.

Arachnoidal Cells

Arachnoidal cells will appear as clumps of cells with poorly defined cell boundaries containing oval nuclei with stippled chromatin.

Cytological appearances of various lesions of brain are discussed based on references from Stephen et al 2001 and Shanop et al 1999. This would help us immensely in differentiating the various pathological lesions of the Central Nervous System.

Reactive changes

It is important that an intense reactive change should not be misinterpreted as neoplastic process. In any reactive process in the brain and in areas of edema around the tumor the characteristic blue color of the neurophil tends to be paler than in normal brain. Numerous large reactive astrocytes with elongated slender cytoplasmic processes and proliferating capillaries would readily mimic tumors. The differentiating features from a Glioma would be hemosiderin pigment and inflammatory cells which are absent in Glioma.

Large Reactive Astrocytes

Large Reactive Astrocytes appear as large fibre forming astrocytes seen adjacent to capillaries with well defined cell margins and cell processes. Some have poorly defined processes and cell margins. Nuclei of gemistocytic astrocytes will be pale with well defined nucleolus. It could be confused with small neurons.

Lipid phagocytes

Lipid phagocytic cells will be round to oval with foamy cytoplasm and small darkly staining nuclei. They will appear larger in smears than in tissue sections because they are usually flattened in the course of preparing the smear.

Abnormal Microglia

a. Abnormal Microglia containing Hemosiderin or Hematoidin will be seen.

b. Hypertrophied elongated type known as red cell will be seen in viral encephalitis.

c. Reactive process will be accompanied by proliferation of endothelial cells and capillaries. Capillary proliferation of extreme intensity would produce so called glomeruloid structures seen in anaplastic tumors.

Mitotic Figures

Typical ones will be seen in microglia and capillary endothelial cells while atypical aberrant mitotic figure would be seen in reactive astrocytes.

Polymorphonuclear leucocytes

Polymorphonuclear leucocytes will be seen during an acute inflammatory process, recent infarction or in response to injury. Perivascular cuffing by lymphocytes and plasma cells is also easy to identify in smears. Such cuffing may be present adjacent to cerebral tumors and also in viral encephalitis and adjacent to brain abscess.

Tumors of Neuroepithelial Tissue

Astrocytic Tumors

Astrocytoma

The Astrocytoma can be of fibrillary, protoplasmic, or gemistocytic type. The fibrillary tumors because of their firm consistency are difficult to smear.(Shanop et al 1991). They would have interlacing fibrillary processes and scant ill-defined cytoplasm. There is little nuclear pleomorphism. The nuclei are oval and have a stippled chromatin. The neoplastic astrocytes tend to cluster around blood vessels giving a distinct papillary appearance. The cells in fibrillary Astrocytoma appear to be elongated. To some degree this elongation could be due to smear artifact. The cellularity of the smear in other types is about two to three times that of normal white matter.

It is sometime difficult to distinguish between protoplasmic and fibrillary Astrocytomas in smears. If there is a prominent gemistocytic component, the astrocytes would appear as oval or round cells with abundant pale homogenous cytoplasm and eccentric nucleus. These cells would be larger than reactive astrocytes, occur in sheets, have larger nuclei and would be devoid of cell processes. There will be no necrosis, vascular proliferation or abnormal mitotic figures. Frequently neurons and calcospherites will also be seen in the tumor.

Anaplastic Astrocytoma

In Anaplastic Astrocytoma the smear would have an uneven pattern and the tumor cells tend to aggregate around small blood vessels. Smears will be moderately to highly cellular and would show a diffusely fibrillary and finely granular background. An increased number of blood vessels show mild to moderate endothelial proliferation and relatively frequent branches. Necrosis is usually absent. The tumor cells will be moderately pleomorphic. Gemistocytic cells may be a significant component. The neoplastic cells would have more atypical nuclei with accentuated angulation of the nuclear outline and coarse chromatin.

Glioblastoma

In Glioblastoma the tumor tissue would spread fairly evenly usually with a tendency of the tumor cells to cluster around blood vessels. The smears will be highly cellular and would show a fibrillated background with necrotic debris, ghost cells, marked endothelial proliferation and vascular glomeruloids. The vascular glomeruloids would appear as well circumscribed three dimensional clusters of irregularly oriented spindle cells. The tumor cells would be large and show mild to marked pleomorphism. Combination of elongated polar- shaped cells, often with an oxyphilic cytoplasm (neoplastic gemistocytes), small cells with naked small hyperchromatic nuclei and bizarre tumor giant cells will be evident.

Giant cell Glioblastoma, a variant of Glioblastoma will occur usually in the cerebrum but can occur at other sites also. It contains many bizarre huge multinucleated giant cells.

Pilocytic Astrocytoma

In Pilocytic Astrocytoma the tumor may be firm and resists spreading. In spite of the smears being very uneven they appear cellular due to tight clustering of tumor cells. Trabeculae of elongated bipolar cells would be seen characteristically surrounding cellular foci containing smaller and

rounded cells with ill-defined cell membranes. Rosenthal fibers and granular bodies will be seen. The background staining would be metachromatic in smears stained with Toludine blue.

Pleomorphic Xanthoastrocytoma and Subependymal Giant cell Astrocytoma

Pleomorphic XanthoAstrocytoma would be characterized by lipid laden pleomorphic neoplastic astrocytes with bizarre multinucleated cells. Mitosis, necrosis and vascular proliferation will be usually absent.

Subependymal giant cell Astrocytoma would be composed of groups of large cells with abundant cytoplasm and eccentric nuclei separated by smaller elongated cells.

These neoplasms are rarely encountered and it seems unlikely that the diagnosis could be made on smears alone.

Gliosarcoma

In Gliosarcoma the tumors may be difficult to diagnose on smears because the tissue does not spread easily. Cytological features would be typical of Glioblastoma. Sarcomatous cells would form thick and tight fascicles. They would have oval to slightly hyperchromatic naked nuclei

embedded in a homogenous pale oxyphilic or fibrillated matrix. Morantz et al (1976) reported that 8% of Glioblastomas are Gliosarcomas.

Oligodendroglial tumors

In Oligodendroglial tumors the tissue usually crushes easily. They form evenly spread moderately to highly cellular smears. Smear would show discohesive relatively small tumor cell with uniform round slight dark nuclei without nucleoli and a rim of pale staining or abundant oxyphilic cytoplasm (Minigemistocytes). Foci of calcification would be often present. Here the cells will not remain attached to blood vessels. Capillaries will not be very prominent in smears. The appearance would simulate Pituitary Adenomas. Hence it would be essential to know the site.

Anaplastic Oligodendroglioma

It would be difficult to diagnose Anaplastic Oligodendroglioma on smears unless the smear shows features of typical Oligodendroglioma along with Anaplastic areas.

Ependymoma

In Ependymoma the smear would show tumor cells that are small, uniform, round to oval with clefted nuclei and adhere in blood vessels to

form perivascular pseudorosettes. Pseudopapillary clusters would be seen surrounding blood vessels in papillary variant.

Anaplastic (malignant) Ependymoma

In Anaplastic (malignant) Ependymoma the smear would show the basic cell type and architecture along with numerous mitotic figures and necrosis. The site of the tumor would also help to arrive at an accurate diagnosis.

Myxopapillary Ependymoma

Myxopapillary Ependymoma occurs in the region of the cauda equina and in the filum terminale. Smear would show typical papillary pattern. The presence of stellate cells in metachromatic mucoid matrix can give suspicion of chordoma. The site of the lesion will lead to accurate diagnosis.

Subependymoma

Subependymoma would be difficult to smear probably because of its firm consistency. Here frozen sections are preferable to smears. The smear would show clumps of cells within a dense fibrillary stroma.

<u>Mixed Glioma</u>

Mixed Oligo Astrocytoma and Mixed Oligoependymoma

It may be difficult to diagnose on smear alone. The tumor would be suspected if both components are seen on the smear.

Choroid Plexus Tumors

Choroid plexus Papillomas

In Choroid Plexus Papilloma the smear would show delicate papillae, presence of cuboidal cells on the surface which can be single layered or 2 to 3 layers thick with capillaries. The papillary pattern is so striking that it is possible in smears to distinguish between Choroids Plexus Papilloma and Ependymoma.

Anaplastic Choroid Plexus Papilloma

In Anaplastic Choroid Plexus Papilloma the smear would show prominent papillary pattern along with abnormal mitotic figures and necrosis. These are rare tumors..

Neuroepithelial tumors of uncertain origin

These are very rare tumors and are difficult to diagnose on smears alone.

Neuronal and mixed Neuronal glial Tumors

Ganglioglioma

In Ganglioglioma the smear would show large aberrant ganglion like cells and neoplastic astrocytes. Often these cells would be interpreted as giant cells in an Anaplastic Astrocytoma or Giant Cell Glioblastoma.

Central Neurocytomas

In Central Neurocytoma the smear would be moderately cellular and will be composed of neoplastic cells which are round with clear cytoplasm and well defined cell membrane. In smear preparations it would closely resemble an Oligodendroglioma.

Pineal Parenchymal Tumors

In Pineal Parenchymal tumors the tumor tissue would spread evenly to form highly cellular smears. The smear will show cells which are round to oval with moderate amount of oxyphilic cytoplasm, round nuclei and coarse chromatin. The background would be finely granular. A proper history correlating with neuroradiological findings would help to clinch the diagnosis.

Embryonal Tumors

Medulloblastoma

In Medulloblastoma the smear would spread easily. The smear will show evenly distributed sheets of closely packed tumor cells. The cells will be

round to slightly elongated. They will have small amount of dark staining cytoplasm and hyperchromatic nuclei. Rosettes will be seen in the well differentiated type but would be poorly formed in the poorly differentiated type. Tumor cannibalism, target inclusions, cytoplasmic vacuoles and prominent multiple nucleoli would be noticed frequently in the undifferentiated type. (Kumar PV et al 2002) . There would be variable mitotic activity. Background will be finely granular and occasionally may be necrotic. Occasionally Purkinje cells may be seen mixed with the granule cells. Heterologous cells such as rhabdomyoblasts, melanoblasts, melanocytes and even mature fat cells may be present. Desmoplastic Medulloblastoma being very firm resists spreading and would be rarely identifiable in smears.

Tumors of Nerve Sheath

It would be difficult to differentiate between Schwannomas and Neurofibromas even in paraffin section

Schwannoma

In Schwannoma the smear will be soft and creamy and would show predominantly spindle shaped Schwann cells. One of the features at low magnification would be the presence of many discrete groups of cells of

varying size. At higher magnification Antoni type A areas composed of trabeculae of elongated spindle cells would be seen. Loosely cellular type B areas would show large cells with ill-defined cytoplasm and cell boundaries. No mitotic figures would be seen. Hemosiderin is often present but palisading and lipid containing cells will not seen in the smear.

Neurofibroma

In Neurofibroma, the smear would show a mixture of Schwann cells and fibroblasts. Smears would be difficult to make and will show a tumor of low cellularity. Cells would be characteristically spindle shaped and would lie in a pale staining matrix. Malignant Schwannomas will not be identifiable in smears. Recurrent neurofibromas may be highly cellular and contain numerous mitotic figures.

Tumor of the Meninges

Meningiomas

Meningiomas arise from the arachnoid and most types would have some common features in smears. The smear would show tumor cells arranged in discrete irregularly shaped clumps of varying sizes. At higher magnifications, the smear would show cells with a moderate amount of pale cytoplasm, poorly defined cell boundaries and vesicular nucleus with chromatin dots and sometimes nucleoli. The most characteristic diagnostic feature would be the presence of cell whorls. These would be numerous in Transitional and Psammomatous Meningiomas. Psammoma bodies though less common than whorls are

more numerous in Spinal Meningioma. Glove powder could be mistaken for psammoma bodies.

In smears whorls will not be seen in Fibrous and Hemangiopericytic type and hence one would have to be acquainted with the appearance of arachnoidal cells to interpret these lesions. In Hemangioblastic meningioma the smear would show considerable cellular pleomorphism with pale staining vacuolated cytoplasm and hyperchromatic nucleus, but only rarely will arachnoidal cells and occasional cell whorls be seen.. There would be no mitotic figures seen in this type. Metastatic Renal Carcinoma would show similar findings and at times may be difficult to differentiate from Hemangioblastoma. Hemangiopericytic Meningioma would show highly cellular smear, often with distinct papillary appearance and oval hyperchromatic nuclei separated from each other by very scanty cytoplasm. Mitotic figures will be seen.

Fibrous Meningioma would be difficult to diagnose in smears because the tissue being firm resists spreading. The smear would show numerous trabeculae with groups of elongated cells.

Intraventicular Meningiomas would also be difficult to diagnose on smears because the cells will be of fibrous type and would simulate Fibrillary Astrocytoma.

Another non specific feature which would help in the diagnosis of Meningioma would be the presence of scattered mast cells containing intracytoplasmic metachromatic granules. These mast cells will be seen mostly in Hemangioblastic and Hemangiopericytic types of Meningioma. Cellularity, hyperchromatism of the nuclei and numerous mitotic figures could lead to the suspicion that the tumor is malignant.

Malignant Lymphomas

In Malignant Lymphoma the smear spreads easily and would show highly cellular non-cohesive small lymphoid cells with distinct cytoplasmic borders and mild nuclear pleomorphism admixed with reticulin cells. These cells would be almost three times larger than red blood cells. They would be seen forming concentric rings around blood vessels. Reed Sternberg giant cells would also be seen in Hodgkin's Lymphoma.

Germ Cell Tumor

Germinomas would spread easily to form highly cellular smears in a clean background with inconspicuous blood vessels. The smear would show two types of discohesive cells, large neoplastic cells and small mature T Lymphocytes. The large spherical to polygonal tumor cells would have distinct cell membrane with clear to oxyphilic glycogen containing cytoplasm centrally located large vesicular nuclei, granular chromatin and prominent basophilic nucleoli. Mitotic activity may be conspicuous. Syncytiotrophoblastic giant cells may be present.

Cyst and Tumor like Lesions

Epidermoid Cyst

Epidermoid Cyst would show anucleate squames in a granular background.

Other cystic lesions are Colloid cyst of third ventricle, Enterogenous cyst.

Tumors of the Sellar region

Craniopharyngiomas

Craniopharyngiomas may not smear well and so a wet film from any cyst fluid would be more helpful, as it contains many cholesterol crystals in the fluid. The smear would show sheets of transitional or stratified

squamous cells forming honeycomb pattern with or without palisading row of cells. Papillary clusters, squamous cell clusters, anucleate squames, calcification, glandular or rosette like structures, ciliated columnar cells, spindle cells and multinucleate giant cells may be seen occasionally. (Daneshbod 2005 et al) .. Sometimes the adjacent brain would show features of Gliosis similar to Fibrillary Astrocytoma.

Pituitary Adenoma

In Pituitary Adenomas, the smear would show sheets of oval or round cells with occasional papillary pattern in the background. Most of the cells would be moderately pleomorphic with varying amount of pale staining cytoplasm conspicuous nuclei and nucleoli. Binucleate and multinucleate cells with nuclear pleomorphism would also be seen commonly in these smears. Some smears would be composed entirely of smaller more uniform cells.

Chromophobe Adenomas would be less pleomorphic than Prolactinomas or Eosinophilic Adenomas. It would be difficult to differentiate normal parenchyma from Pituitary adenoma. The normal parenchyma would smear less easily and hence there would be more clumping of cells. Smears from infundibular process (posterior lobe) would show closely packed interwoven elongated cells.

Metastatic Tumors

Many tumors form secondary deposits in the brain. Hence, brain would have diverse type of tumors. There would be a sharp transition between tumor tissue and brain. Tumor cells would spread out evenly and

individually so that they have clear outlines, distinct epithelial appearing nuclei, conspicuous nucleoli and a pale nucleoplasm.

Small cell carcinoma of lung would contain numerous mitotic figures, bizarre giant cells and foci of necrosis rendering it difficult to differentiate from Anaplastic Astrocytoma.

In some Mucin Secreting Adenocarcinomas, considerable amount of metachromatic material may be seen among tumor cells.These smears could be stained for mucin.

In malignant melanoma the smear would show abundant intracellular and extracellular slate grey colour granules of pigment.. True nature of these pigments could be established by special stains.

Tumors of Blood Vessel origin

Hemangioma

In Hemangioma the smear would be thick and does not spread well. Abnormal vessels would be seen with hemosiderin deposits. This tumor is not recognized in the revised WHO Classification of CNS Tumors.

Hemangioblastoma

Hemangioblastoma is a tumor of vasoformative cells and is restricted to the cerebellum and brainstem. These smears would not smear easily. Smear would show thick and thin dense trabeculae of closely packed elongated cells, clear areas in between would show pale staining and slightly twisted nuclei along with numerous mast cells and hemosiderin containing phagocytes.

Lesions compressing the Spinal Cord

Lesions compressing the spinal cord may be intramedullary, intradural extramedullary or extradural. An intramedullary tumor may be a neuroepithelial tumor whereas an intradural extramedullary tumor may be a Meningioma or Peripheral Nerve Sheath tumor. Extradural tumors will be Metastatic carcinoma, Lymphoma and Plasmacytoma.

Myeloma

In Myeloma the tissue would smear out easily, cells spread out individually, smear would stain deeply and plasmacytic nature would be discernible.

Granulomas and Tuberculomas

These smears would show epitheloid cells, Langhans type giant cells and non specific infiltration by small mononuclear cells.

Chordoma

In Chordoma the smear would shows scattered cells with distinctly vacuolated cytoplasm in a sea of strongly metachromatic matrix

Glomus Jugulare Tumor

Glomus Jugulare Tumor is present at the cerebellopontine angle and correct diagnosis could be made from crush preparations..

Other types of Lesions

Encephalitis and Infarction are other types of CNS Lesions that could be diagnosed by smears.

Encephalitis

In Encephalitis the smear would have to be taken from an appropriate site to facilitate easy diagnosis of the lesion. Smear would show numerous vessels cuffed by lymphocytes and plasma cells. The zone adjacent to the small vessels would be intensely cellular due to the presence of lymphocytes, plasma cells, hypertrophied microglia and polymorphs.

In some cases, toxoplasma pseudocysts could also be identified in smears.

Cerebral Infarction

In Cerebral Infarction the smear would show the presence of lipid phagocytes, reactive astrocytes and proliferating capillaries. The

reaction would be so intense that one could mistake it for a malignant tumor.

Lesions in Bone

Crush preparations would help in diagnosing Bone Lesions. Aneurysmal bone cyst, Eosinophilic granuloma of the skull, Fibrous dysplasia, Osteogenic sarcoma, Chondrosarcoma could be diagnosed with the help of smears.

Classification and Grading of Central Nervous System Tumors

The tumors of the Central Nervous System arise from cells derived from the primitive neuroepithelium. Most classifications have reflected a general acceptance of the histological tumor types by linking neoplastic elements to normal cell types found in the mature and developing nervous system.

The present day concept of classification of the tumor was initiated by R. Virchow(1863). He classified these tumors for the first time according to the cell type. He provided one of the earliest description of what we term a Glioblastoma Multiforme.

Earlier workers like Tooth(1912) have emphasized the correlation of morphological structures and clinical course in the first extensive histological study of neurosurgical material. He followed Cohnherms theory that tumors develop from embryonic rests.

Most modern classifications are based upon that of Bailey and Cushing (1926). These authors studied the embryogenesis of the various cellular components of Central Nervous System. Then they attempted to classify the tumors observed in terms of the different morphological stages through which these cells pass in oncogenesis using metallic techniques to supplement routine staining methods.

Having enumerated 20 cell types they proposed a classification based on a pyramidal scheme of cytogenesis using 14 main groups as follows

1. Medulloepithelioma
2. Medulloblastoma
3. Pineoblastoma
4. Pinealoma
5. Ependymoblastoma
6. Ependymoma
7. Neuroepithelioma

8. Spongioblastoma
    a. Multiforme
    b. Unipolare
9. Astroblastoma
10. Astrocytoma
    a. Protoplasmaticum
    b. Fibrillare
11. Oligodendroglioma
12. Neuroblastoma
13. Ganglioneuroma
14. Papilloma Chorideum

Subsequent to the publication of the classification of Bailey and Cushing increasing attention was directed to the frequency with which anaplasia determined the morphological characteristic of tumor cell populations and their diversities in malignant Gliomas. This invited a reappraisal of those constituent cells that are structurally reminiscent of primitive embryonal types.

Influenced by such considerations, Kernohan and Sayre (1949) swung the pendulum hard in the direction of simplification by introducing a

system of grading from 1 to 4 in ascending order of malignancy. They reduced the Glioma types to 5 main groups. This resulted in the elimination of several tumor entities including the Glioblastoma Multiforme.

Glioma Classification According To Degree Of Malignancy

(Kernohan and Sayre)

New	Old (with new in parentheses)
Astrocytoma Grade 1-4	Astrocytoma (Astrocytoma Grade 1) Astrocytoma (Astrocytoma Grade 2) Polar Spongioblastoma (Obsolete) Glioblastoma Multiforme (Astrocytoma Grade 3 & 4)
Ependymoma Grade 1-4	Ependymoma (Ependymoma Grade 1) Ependymoma (Ependymoma Grade 2 & 3) Neuroepithelioma (Obsolete) Medulloepithelioma (Ependymoma Grade 4)
Oligodendroglioma Grade 1-4	Oligodendroglioma (Oligodendroglioma Grade 1) Oligodendroglioma (Oligodendroglioma Grade 2-4)
Neuroastrocytoma Grade 1-4	Neuroastrocytoma Grade 1 Neurocytoma Ganglioneuroma Gangliocytoma Ganglioglioma Neuroastrocytoma Grade 2-4

	Neuroblastoma Spongioneuroblastoma Glioneuroblastoma Others
Medulloblastoma	Medulloblastoma

Through succeeding years existence of a number of rare Gliomas, previously proposed as embryonal, had been called into question by virtue of modern ultrastructural immunohistological and molecular genetic investigations.

The international classification of CNS tumors, drafted under the auspices of WHO (Zulch 1979) represented the first consensus achieved after many years of study and discussion by proponents of different schools. Its main contribution was to reconcile the terminologies and concepts of sometimes widely differing viewpoints and thus achieve, by compromise a reasonable measure of uniformity on the most commonly recognized tumor entities and their most frequent variants. This classification was further modified and the current classification is based on WHO 2000 criteria. It is largely based on morphology. Although imperfect, controversial and without molecular basis the

current WHO classification is most widely used system (Classification enclosed in Annexure I).

Grading CNS Tumors

Any scheme of histological grading has two main goals.

1. Tumor grade must predict clinical behavior
2. Grading criteria must be sufficiently objected and defined to minimize variations among observers and to maximize reproducibility (Fulling & Nelson 1984)

In any valid grading scheme of malignancy, representative histological material must have been obtained for evaluation.

Historically the first system for grading Gliomas were represented about the same time (Kernohan et al 1949, Svien et al 1949, Ringertz 1950). All the systems base the histological grade on the recognition of cellular anaplasia.

Kernohan and Svien used a four grade system (Grade 1 through 4) which was largely based on the proportion of normal tissue remaining mixed with the invading tumor and the morphological configuration of the invading edge of tumor into the surrounding normal brain tissue.

The two difficulties in Kernohans grading were the invading edge and the proportion of cellular anaplasia. This contributed to the lack of clinical relevance in separating the individual tumor grades.

Ringertz1950 used the three grade system. He used necrosis as a single feature to divide intermediate type Astrocytoma from Glioblastoma. This had clinical relevance and showed significant difference in survival among patients with different grades. Thus the three tiered grading system was popularized. These three tiered grading systems are hence more closely related with clinical survival and hence more widely used today in diagnostic neuropathology.

These grading systems include

Ringertz(1950)

Nelson (1983)

Fulling and Nelson1984

Burger1985

Burger and Green1987

Daumas and Duport (1988b) also called St Annes Mayo system

Current system is advocated by WHO in its 2000 revision

The St. Anne Mayo scheme detailed by Daumas Duport (et al 1988b) published a simple and reproducible method for grading Astrocytomas. The method under study was for use on ordinary Astrocytoma cells types (ie) fibrillary, gemistocytic, anaplastic and Glioblastoma.

It was based upon the recognition of the presence or absence of 4 morphological criteria

- nuclear atypia
- mitosis
- endothelial proliferation
- necrosis

0criteria - Grade1

1criteria - Grade2

2 criteria - Grade3

3 criteria - Grade4

This grading system clearly distinguished 4 distinct grades of malignancy whereas Kernohan grading system accurately distinguished

only 2 major groups and Ringertz grading distinguished only 3 major groups.

In modified WHO classification 2000, criteria used for grading Astrocytomas (1-4) were similar to those used in Daumas Duport scheme. The essential variance of this system from the other three tiered system was that a tumor can be classified as grade 4 tumor based on the presence of vascular changes and without the feature of necrosis.

Disadvantages of Histological Grading

1. Anaplastic features may be focal and the sample obtained may not be from a representative site.

2. It does not take into account the anatomical location of the tumor which determine surgical accessibility and likelihood of complete resection.

(Details of WHO grading enclosed in Annexure I)

Materials and Methods

Materials and Methods

Biopsy samples collected from the Department Of Neurosurgery, Stanley Medical College and Govt. Stanley Hospital during January 2004 to January 2006 were taken for study. A total of 100 cases were reported during this period. Tissue biopsy of all intracranial space occupying lesion were obtained during surgery in the theatre directly from the surgeon. The tissue was received in a gauze moistened with saline.

The slides were stained immediately and immediate report was given as regard to the adequacy of the sample, with respect to the lesion. If the sample showed only normal tissue, more samples were collected and more smears were prepared. Except four cases in all the other cases the sample was adequate for reporting. Due to surgical complications and early closure adequate sample could not be obtained in all the four cases.

Preparation of Crush Smears

The tissue fragments usually measure no more than 2mm in diameter. A specimen may at times contain two or more tissue fragments. The

specimen was first examined with the help of a magnifier to see whether the specimen appeared necrotic or hemorrhagic. The apparently viable tissue was then placed on the center of a labeled glass slide. A second labeled slide was placed over the first slide on top of the tissue fragment. Then sufficient pressure was applied between the tips of the thumb and index finger to spread the tissue. If the tissue was soft it spread easily. If it was hard and firm it resisted spreading. In such cases smaller tissue fragments were used to prepare the smear.

The two slides were then pulled apart to produce two thin well prepared smears. One smear was immediately fixed in 95% ethyl alcohol for one to two minutes and stained by rapid Hematoxylin and Eosin method. The second slide was air dried and stained using Toludine blue. A third slide was fixed in 95% ethyl alcohol for 15 minutes. The smears were stained as follows and studied.

1. **Rapid Hematoxylin and Eosin method**
    a. Stain in Hematoxylin for 1 minute
    b. Wash in water
    c. Stain in Eosin for 30 seconds
    d. Wash in water
    e. Wash in absolute alcohol
    f. Clear in Xylol

g. Mount in DPX

The entire procedure took 6 to 8 Minutes

2. <u>Toludine Blue method</u>

a. The smear is covered with 1% alcohol solution in Toludine Blue for 1 minute
b. The slide is drained and then rinsed gently with water to remove excess stain
c. Wash in absolute alcohol
d. Clear in Xylol
e. Mount in DPX

Remaining tissue was fixed in 10% neutral formalin. The tissue slices were processed and paraffin blocks were prepared. Thin sections were cut from the paraffin block and stained with Hematoxylin and Eosin as follows and studied.

<u>Routine Hematoxylin and Eosin method</u>

f. Stain in Hematoxylin for 5 minute
g. Wash well in running tap water till sections blue
h. Differentiate in 1% acid alcohol for 5 seconds
i. Wash well in tap water until sections are again blue
j. Stain in Eosin for 1 min
k. Wash in water
l. Wash in absolute alcohol

m. Clear in Xylol
n. Mount in DPX

Necessary photographs were taken and an Analysis of CNS Lesions in Squash preparations with Histological Correlation was then done. Statistical details regarding the age and sex incidence of CNS tumors were also studied.

Observation and Results

Observation and Results

Crush Cytology is a fairly accurate, simple and reliable tool for rapid intraoperative diagnosis of Central nervous system lesions. In an attempt to define the accuracy of crush technique and its clinical usefulness, cases received at Neurosurgery Department, Stanley Medical College, Chennai from January 2004 to January 2006 were studied and compared with histopathology. Totally 100 cases were received during this study period. The age and sex incidence of these tumors were also studied.. Cytodiagnosis of CNS lesions on squash preparation was done and correlated with histopathology.

In the 100 cases studied there were 33 cases of Astrocytoma, 15 cases of Meningioma, 16 cases of Schwannoma, 3 cases of Mixed Glioma, 2 cases of Oligodendroglioma, 3 cases of Medulloblastoma, 2 cases of Ependymoma, 1 case of Desmoplastic Neuroblastoma, 1 case of Primitive Neuroectodermal tumor, 2 cases of Metastatic Carcinomatous deposit, 3 cases of Pituitary Adenoma, 1 case of Craniopharyngiomas, 1 case of Hemangiopericytoma, 1 case of Vascular Neurofibroma, 2 cases of Cavernoma, 1 case of AV malformation, 8 cases of Tuberculoma, 2

cases of Epidermal Cyst, 2 case of Cerebral abscess, 1 case of Lipoma (Table 1)

Table 1: Incidence of Brain Tumors in Present Study

Disease	Incidence
Abscess	2
Astrocytoma	33
AV Malformation	1
Cavernoma	2
Craniopharyngiomas	1
Desmoplastic Neuroblastoma	1
Ependymoma	2
Epidermal Cyst	2
Hemangiopericytoma	1
Lipoma	1
Medulloblastoma	3
Meningioma	15
Metastatic Carcinomatous Deposit	2
Mixed Glioma	3
Oligodendroglioma	2
Pituitary Adenoma	2
PNET	1
Schwannoma	16
Tuberculoma	8
Vascular Neurofibroma	1

Complete correlation with the final diagnosis was achieved in 82% of cases. Diagnostic accuracy increased to 92% when cases of partial correlation mainly due to grading deviations were included. 5% of cases had wrong diagnosis.

Total no of cases 100

Cytological investigation correlated with final diagnosis	92
Complete correlation	82
Partial Correlation	10
(due to failure in grading and typing)	
No Correlation	5
Sampling error	4

The discrepancies obtained on comparing squash with Histopathological diagnosis were studied. 2 cases diagnosed as Meningioma on crush preparation turned out to be Pilocytic Astrocytoma and Mixed Glioma in histopathology. Similarly 2 cases of Astrocytoma on histopathology was interpreted as Lymphoma and Subependymoma on crush preparation. A case of Metastatic carcinomatous deposit on crush preparation was diagnosed as Astrocytoma grade 4 on crush cytology (Table 2).

Table 2: Discrepancies noted when comparing squash with histopathological diagnosis

Crush Diagnosis	Final Histopathological Diagnosis
Lymphoma	Grade IV Astrocytoma
Meningioma – Fibroblastic	Pilocytic Astrocytoma

Sub Ependymoma	Astrocytoma Grade IV
Meningioma	Mixed Glioma (Ependymoma with Astrocytoma)
Astrocytoma Grade IV	Metastatic Carcinomatous Deposit

A comparative correlation between Disease and Diagnosis was evaluated with the help of crush preparation and histopathology. Craniopharyngiomas, Abscess, Ependymoma, Epidermal cyst, Medulloblastoma, Pituitary Adenoma, PNET, Tuberculoma and Vascular Neurofibroma showed 100% correlation between disease and diagnosis. Certain tumors like Hemangiopericytoma and AV malformation showed 100% partial correlation. Partial correlation between disease and diagnosis were seen in cases of Mixed Gliomas. This was mainly due to failure in typing and grading the lesions accurately. (Fig 1)

Figure 1:

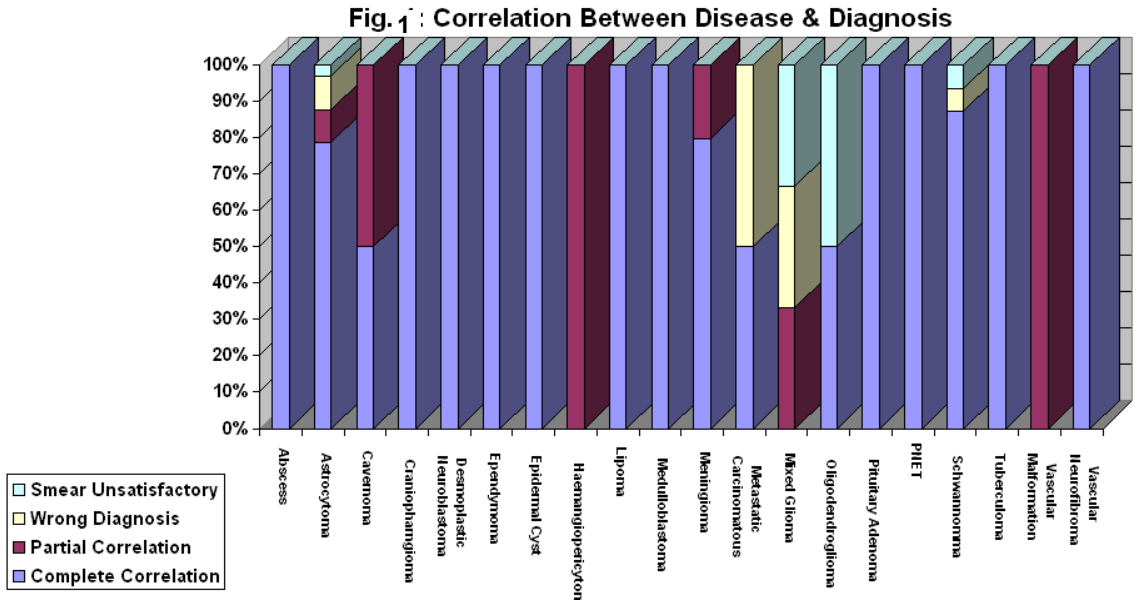


Fig. 1 : Correlation Between Disease & Diagnosis
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Abscess
Astrocytoma
Cavernoma
Craniopharngioma
Heuroblastoma
Desmoplastic
Ependymoma
Epidermal Cyst
Haemangiopeticyton
Lipoma
Medulloblastoma
Meningioma
Metastatic Carcinomatous
Mixed Glioma
Oligodendroglioma
Pituitary Adenoma
PNET
Schwannomma
Tuberculoma
Vascular Malformation
Vascular Heurofibroma
Smear Unsatisfactory
Wrong Diagnosis
Partial Correlation
Complete Correlation

Out of the 100 cases there were 33 cases of Astrocytomas. The smears were cellular and showed the neoplastic astrocytes clustering around blood vessels giving a papillary appearance. Higher grade Astrocytomas like Glioblastomas in addition showed necrotic debris and marked endothelial proliferation. Rosenthal fibers, granular bodies and trabeculae of elongated bipolar cells were seen in Pilocytic Astrocytoma. The bipolar cells surrounded cellular foci containing smaller and rounder cells with ill-defined membranes.

There were 2 cases of Oligodendroglioma. These smears showed discohesive small tumor cells with uniform round dark nuclei. Capillaries were not very prominent in these smears.

There was 1 case of PNET. The smear showed small round cells with scanty cytoplasm. Characteristic rosettes were also seen. There was 1 case of Desmoplastic Neuroblastoma. The smear except for the low cellularity had findings similar to PNET.

There were 15 cases of Meningiomas. The cells were arranged in whorls. The cells had moderate amount of pale staining cytoplasm, poorly defined cell boundaries and vesicular nucleus. In fibrous and

Hemangiopericytic types whorls were not seen. Fibrous Meningioma was composed of numerous trabeculae with groups of elongated cells.

16 cases of Schwannoma were seen. These smears were composed of spindle shaped Schwann cells. Antoni B areas showed large cells with ill-defined cytoplasm and pale boundaries.

There was 1 Case of Vascular Neurofibroma. The smear showed fragments of cohesive spindle shaped cells with eel shaped nuclei along with a few endothelial cells in a myxoid background.

There were 3 cases of Medulloblastomas. The smear showed evenly distributed sheets of closely packed tumor cells which were slightly elongated. Rosettes were also seen.

2 Cases of Ependymoma were seen. The neoplastic cells in these smears were small, round to oval and formed perivascular pseudo rosettes.

3 cases of Pituitary adenoma were seen. The smear showed sheets of round or oval cells. The cells were moderately pleomorphic with variable amount of pale staining cytoplasm, conspicuous nuclei and nucleoli. Binucleate and multinucleate cells were also seen.

There was 1 case of Craniopharnygioma. The smear showed squamous cells and a few multinucleate giant cells.

There were 2 Cases of Cavernoma. The smears showed few strands of endothelial cells with pale cytoplasm and spindle shaped nuclei. Occasional fibroblasts and hemosiderin containing macrophages were also seen.

There was 1 case of Hemangiopericytoma. The smear showed elongated spindle shaped cells fanning out from a vascular core. The nuclei was elongated with granular chromatin and inconspicuous nucleoli. The background was hemorrhagic.

2 Cases of Metastatic Carcinoma were seen. Both the smear showed features of Adenocarcinoma.

There was 1 case of Lipoma. The smear showed mature adipocytes and few fibroblasts.

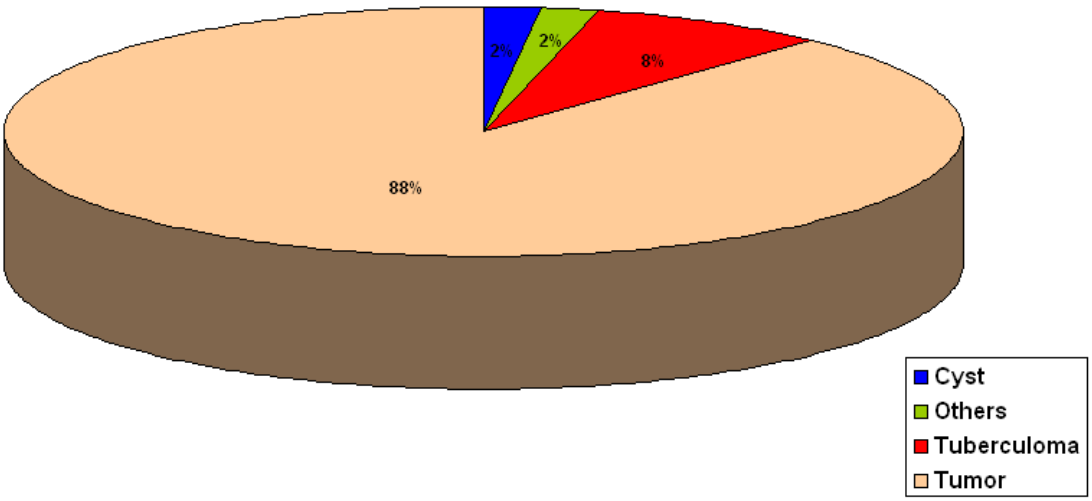
8 Cases of Tuberculoma were seen. The smears showed epitheloid cell clusters in a necrotic background.

2 Cases of Abscess were seen. These smears showed degenerating polymorphs and macrophages.

2 Cases of Epidermoid Cyst were seen. These smears showed debris with anucleate cells, some nucleated squamous cells along with inflammatory cells and foreign-body type giant cells.

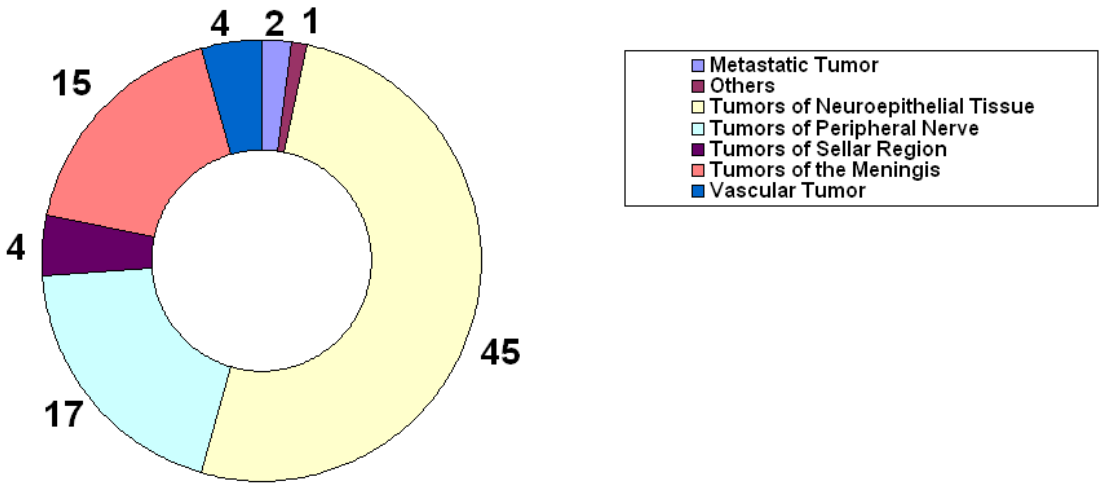
Comparative incidence of Intracranial Space Occupying Lesions studied showed that out of the 100 cases 88% of the cases were tumors. The rest comprised of Tuberculomas 8%, Cyst 2% and Others 2%. (Fig 2)

Fig. 2 Compararative Incidence of Intracranial Space Occupying Lesions



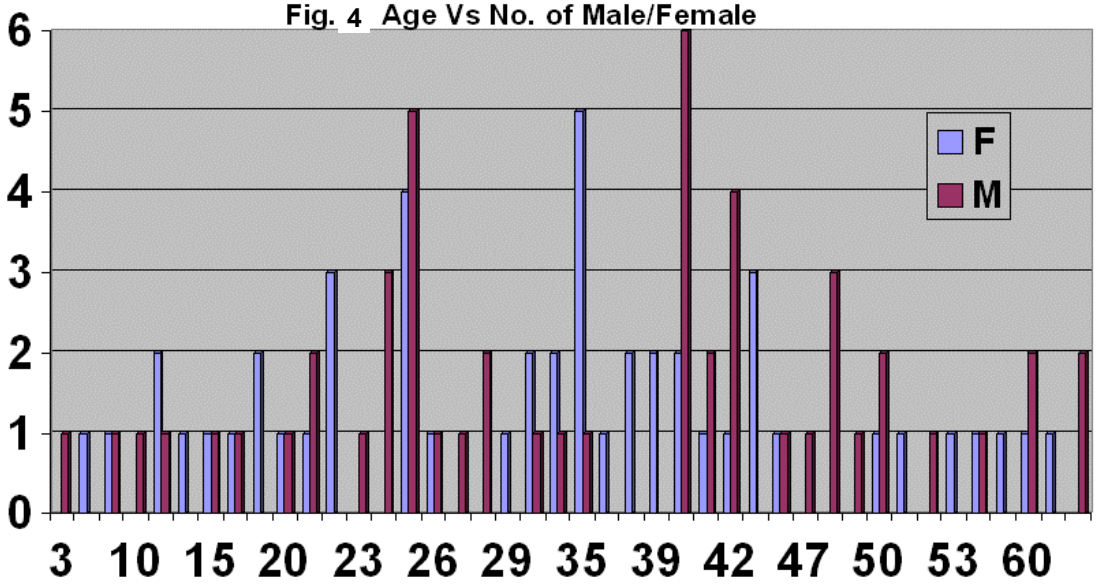
Comparative incidence of brain tumor studied showed that majority of the tumors were of Neuroepithelial origin.(45%). The remaining comprised of tumors of peripheral nerves(17%), tumors of meninges(15%), tumors of sellar region(4%), Vascular tumors (4%), metastatic tumors(2%) and Lipoma(1%). (Fig 3)

Fig. 3 Comparative Incidence of Brain Tumors



The age vs No of males / females were studied. In males maximum of six cases were seen between 39 – 42 years, while in women maximum of 5 cases were seen between 23 – 26 years. Among males the youngest patient was 3 years old while the oldest patient was 70 years old. On the other hand in the case of females the youngest patient was 5 years old while the oldest patient was 65 years old. (Fig 4)

Fig. 4 Age Vs No. of Male/Female



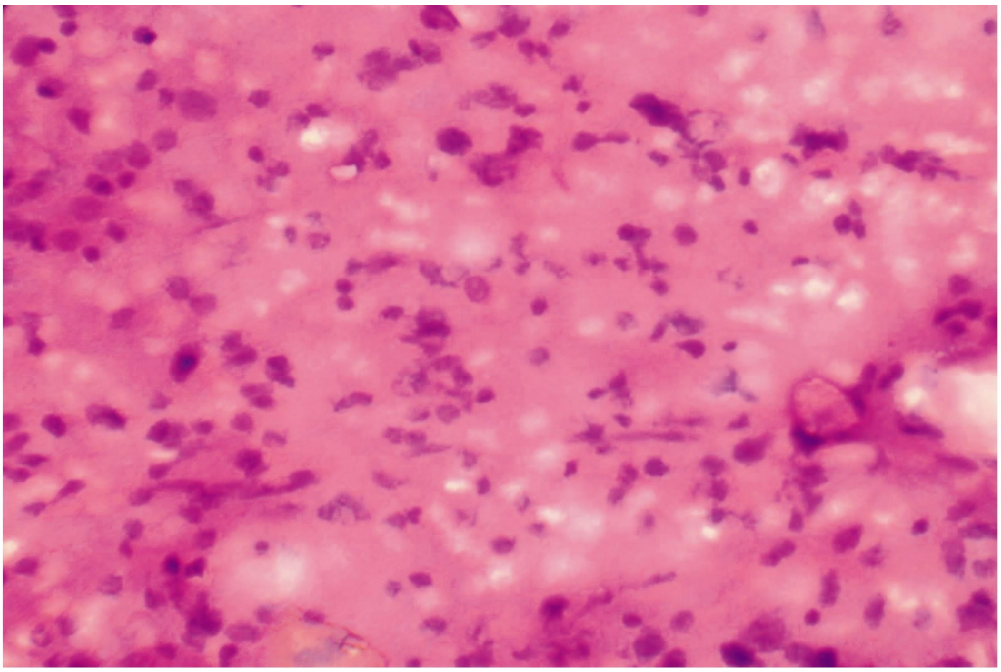


Fig. 1: Low Grade Astrocytoma showing Tumor cells close to blood vessels. Nuclei are oval and cytoplasm is scanty. 50x (H&E)

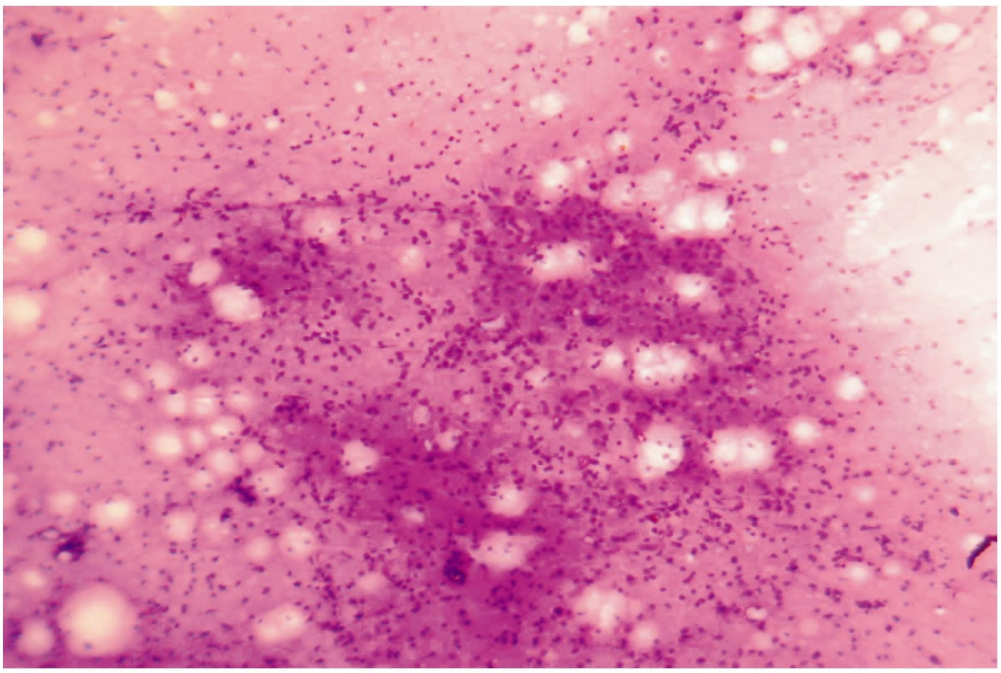


Fig. 2: Low Grade Astrocytoma showing prominent vascularity, fibrillary matrix. Nuclei are oval and cytoplasm is scanty. 200x (H&E)

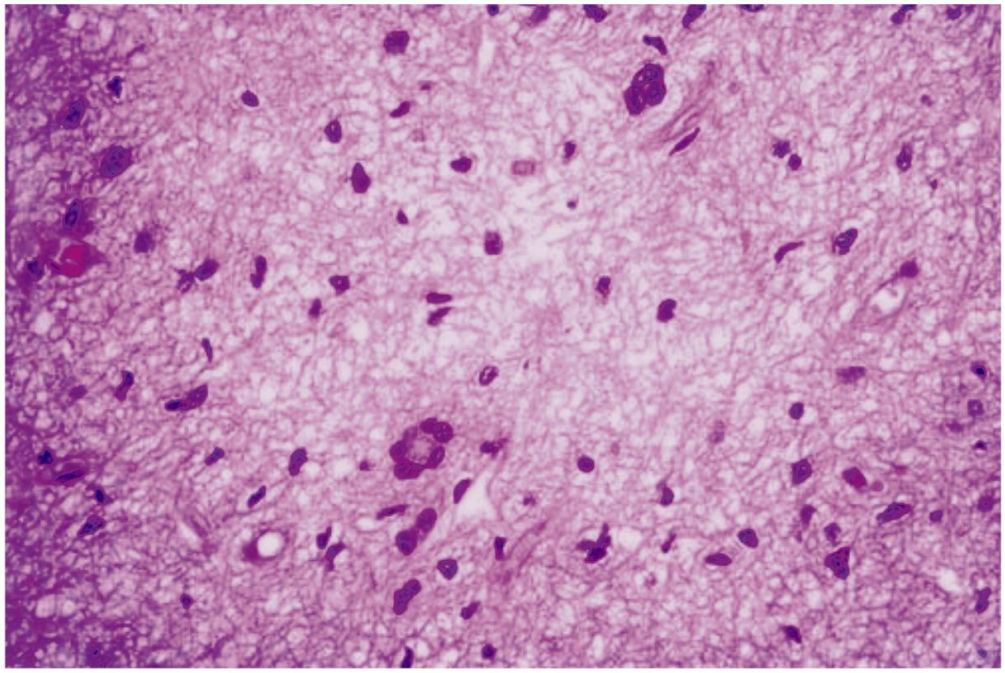


Fig. 3: Low Grade Astrocytoma showing neoplastic astrocytes in fibrillary background. 200x (H&E)

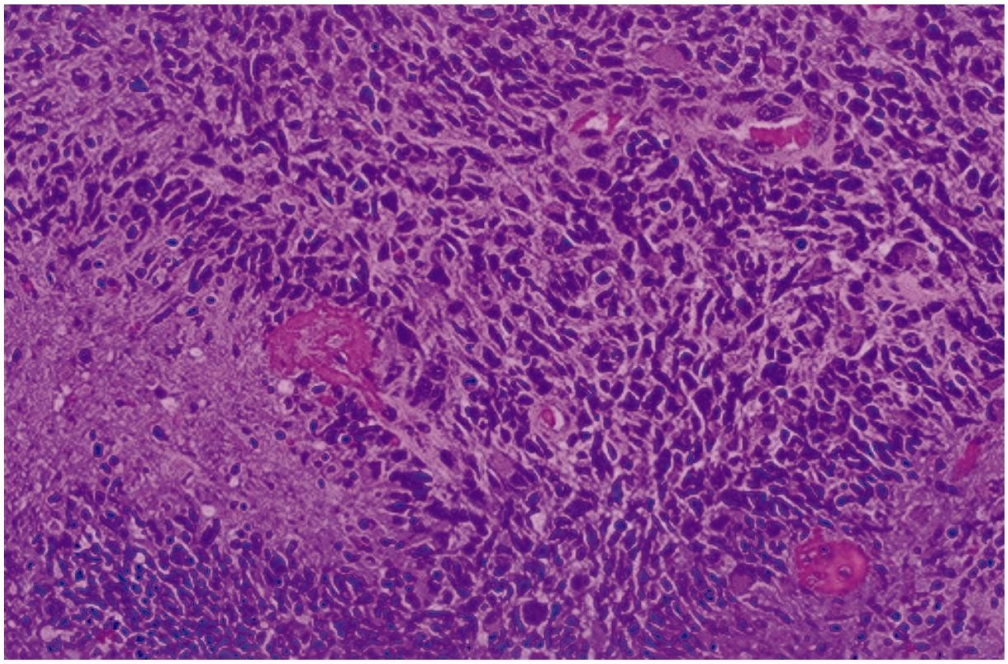


Fig. 4: Glioblastoma Multiforme showing marked cellularity with hyperchromatism, pleomorphism, prominent vascularity and area of necrosis with neoplastic cells palisading around it. 200x (H&E)

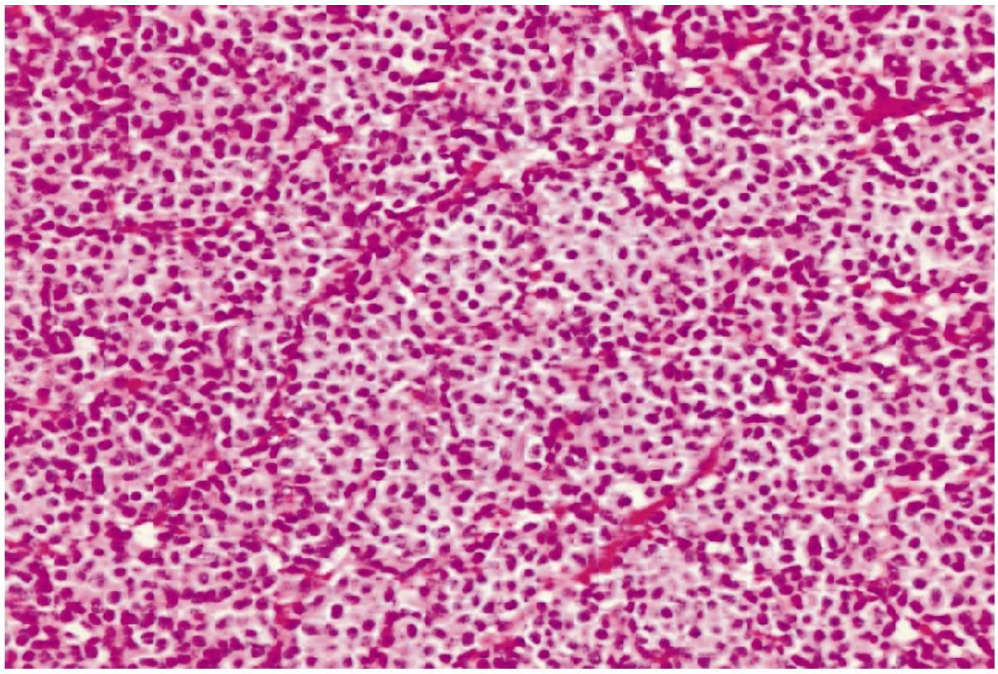


Fig. 5: Oligodendroglioma showing tumor cells with round nuclei and clear cytoplasm. Network of branching capillaries are also seen. 50x (H&E)

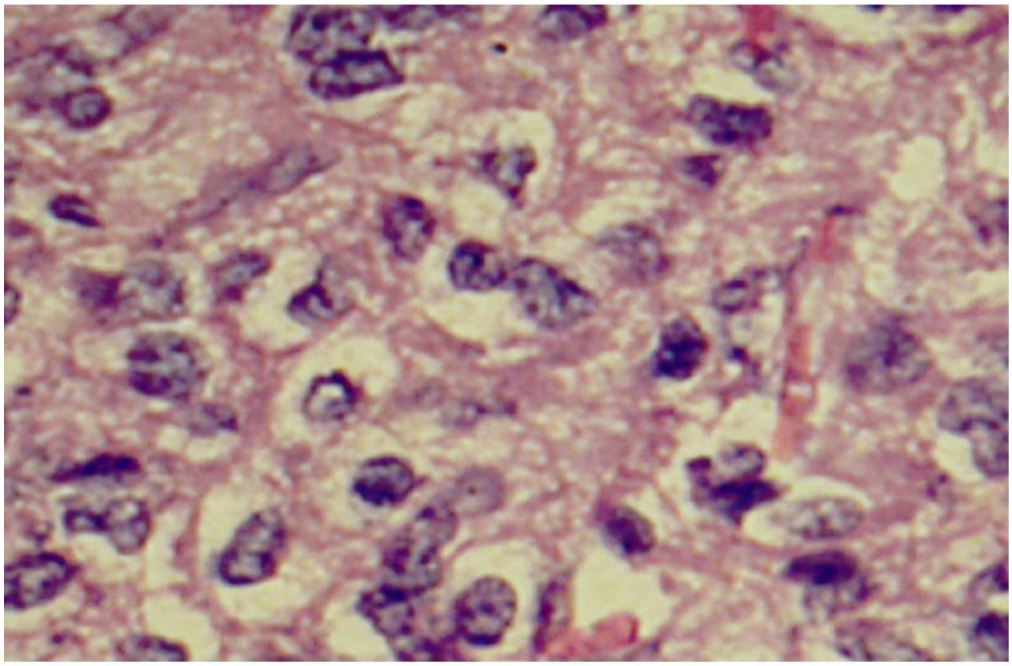


Fig. 6: Oligodendroglioma showing tumor cells with round nuclei and clear cytoplasm. Network of branching capillaries are also seen. 200x (H&E)

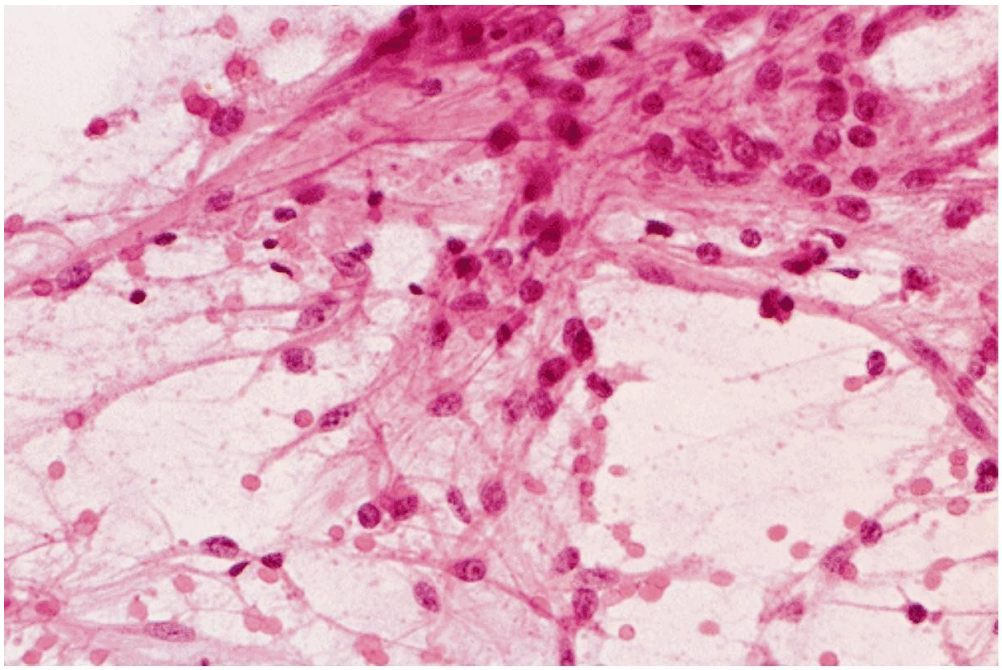


Fig. 7: Pilocytic astrocytoma with elongated cytoplasmic processes. 200x (H&E)

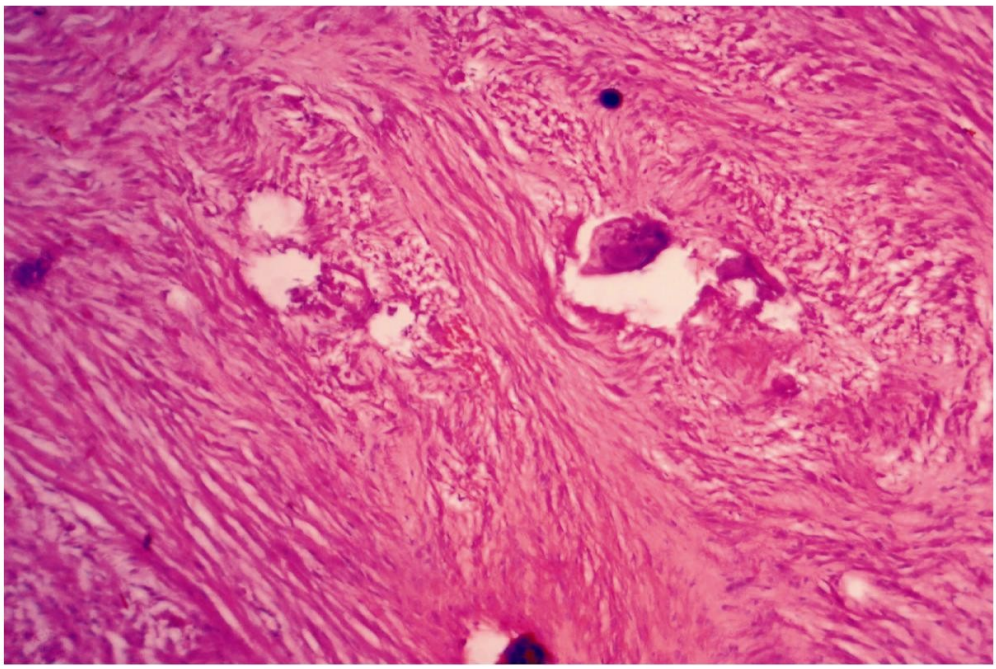


Fig. 8: Pilocytic astrocytoma exhibiting biphasic pattern with compact bipolar cells and cystic areas with calcification. 50x (H&E)

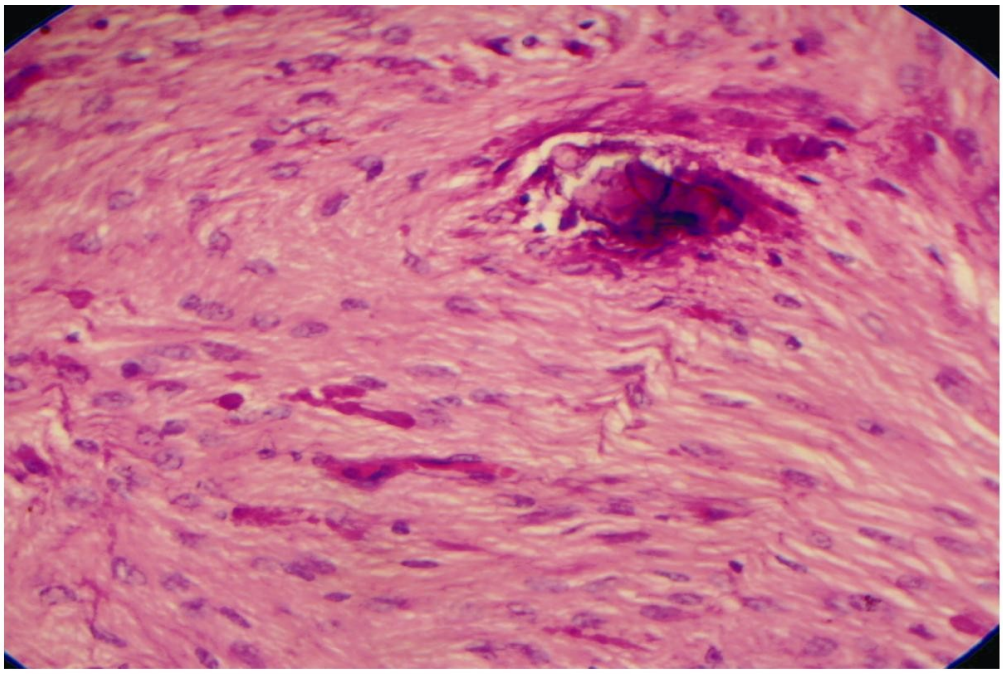


Fig. 9: Pilocytic astrocytoma showing large number of Rosenthal fibres, hyaline globules and calcification. f200x (H&E)

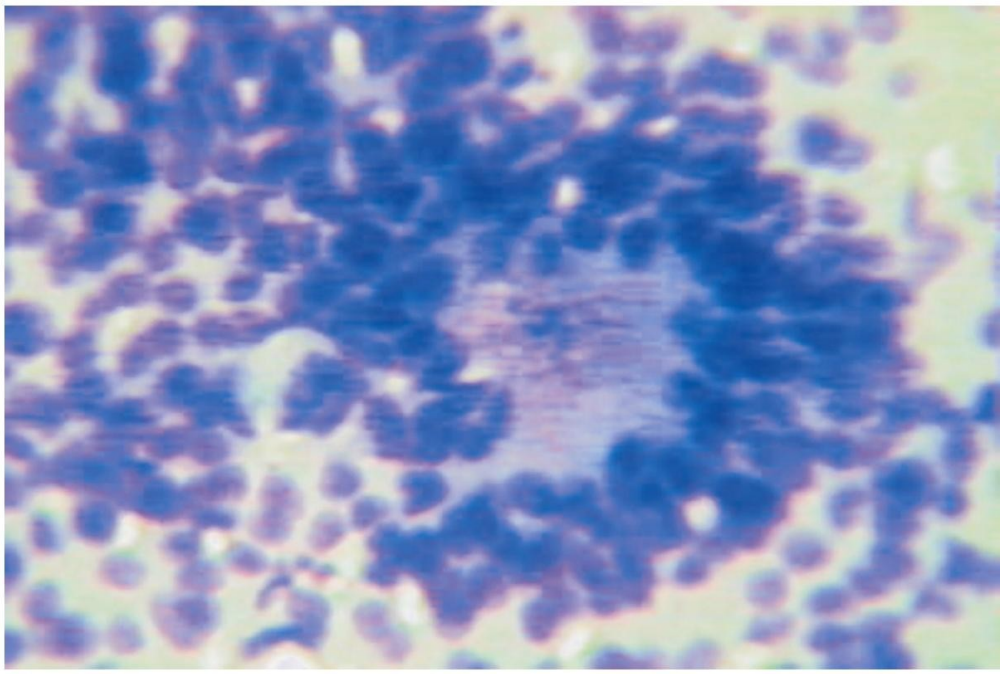


Fig. 10: Ependymoma showing neoplastic cells disposed off in Rosettes. 200x (Toludine Blue)

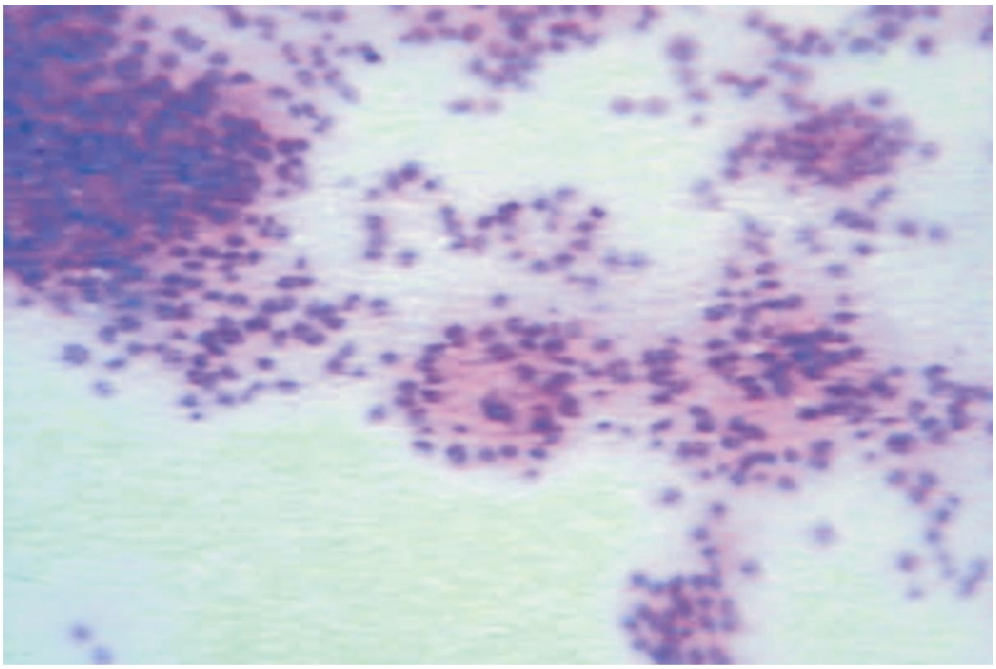


Fig. 11: Ependymoma: Cells smear out individually and have an epithelial appearance. Numerous Rosettes are also seen. 50x (H&E)

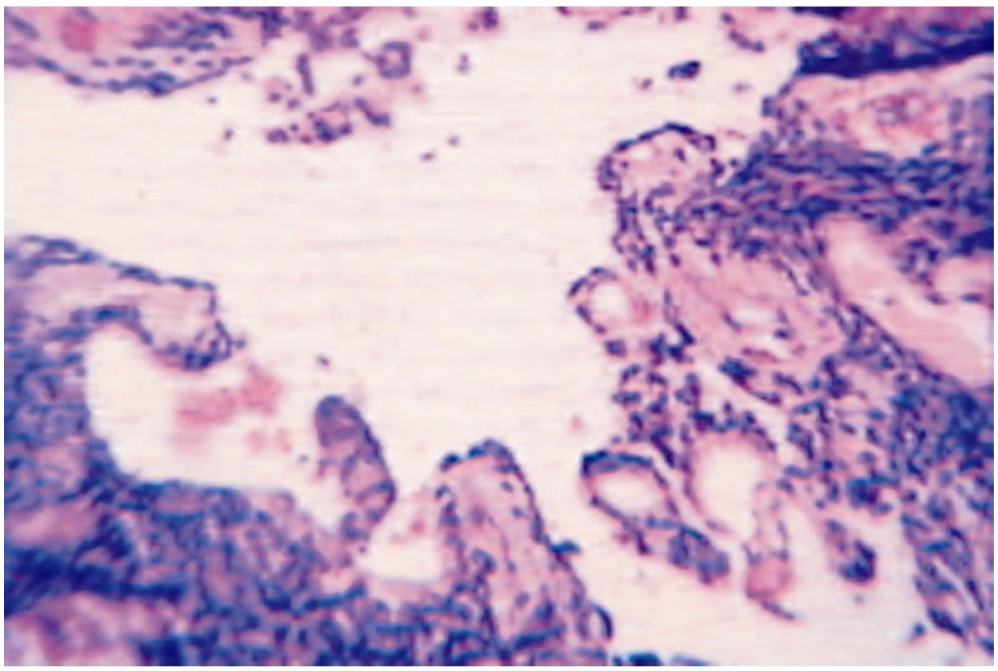


Fig. 12: Ependymoma showing neoplastic cells which are round to oval with perivascular pseudorosettes. 50x (H&E)

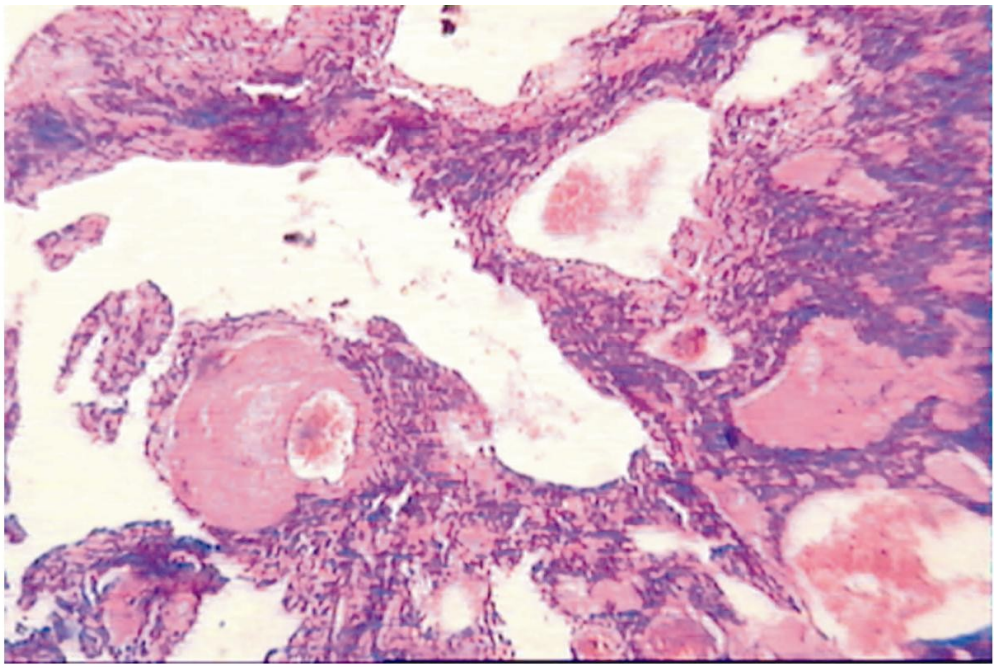


Fig. 13: Ependymoma showing neoplastic cells which are round to oval with perivascular pseudorosettes. 50x (H&E)

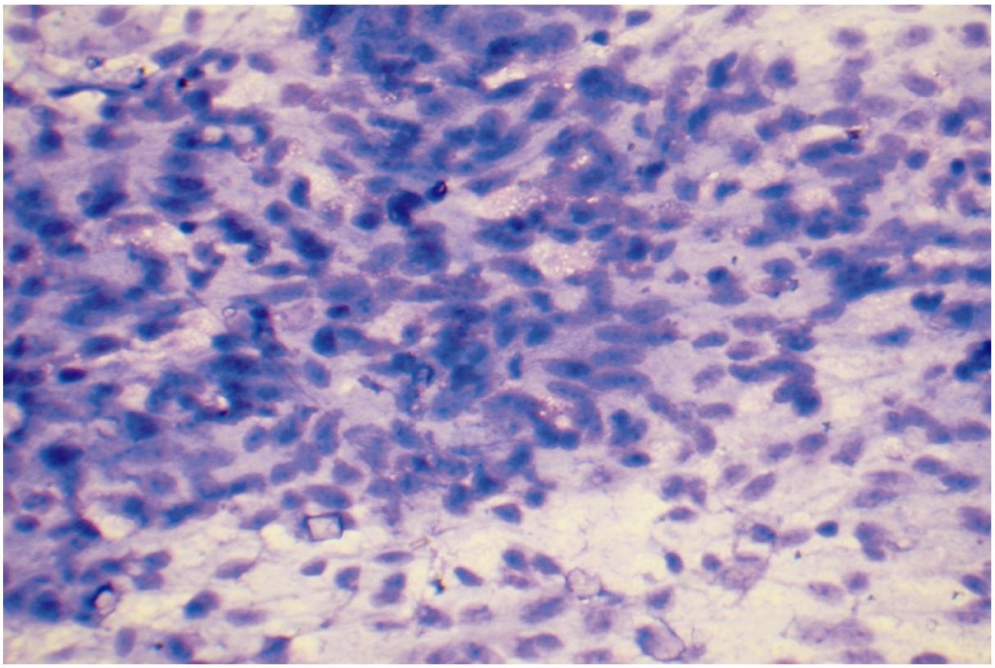


Fig. 14: Medulloblastoma showing closely packed tumor cells with elongated carrot shaped nucleus and forming rosettes. 200x (Toludine Blue)

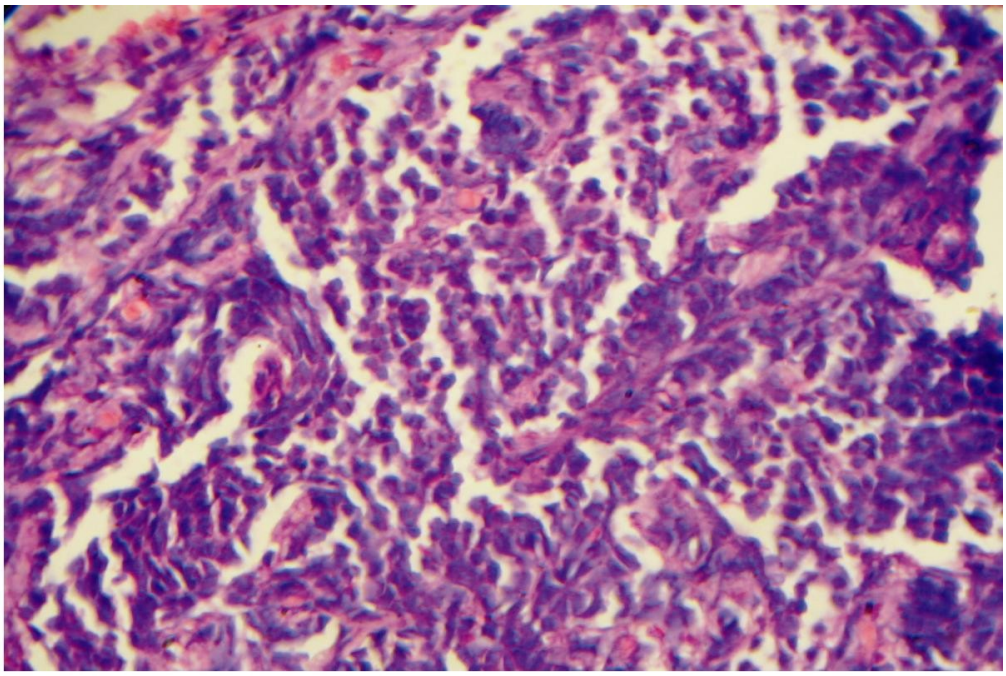


Fig. 15: Medulloblastoma showing closely packed tumor cells and Homer Wright Rosettes. 200x (H&E)

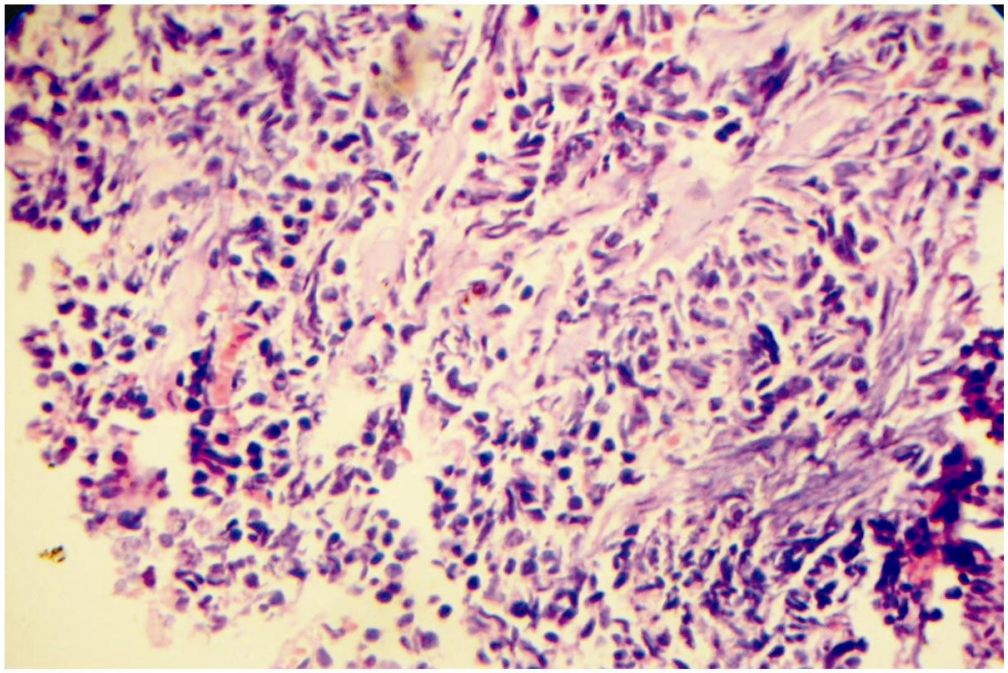


Fig. 16: Medulloblastoma showing closely packed tumor cells and Homer Wright Rosettes.200x (H&E)

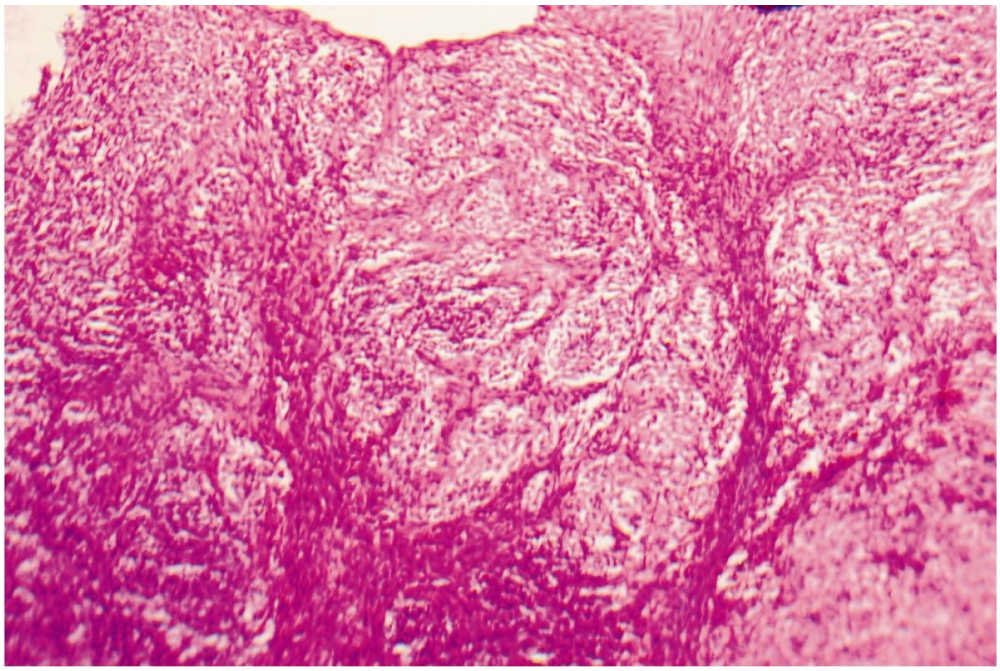


Fig. 17: Desmoplastic Neuroblastoma. Micronodular zones of reduced cellularity (Pale Islands) seen. 50x (H&E)

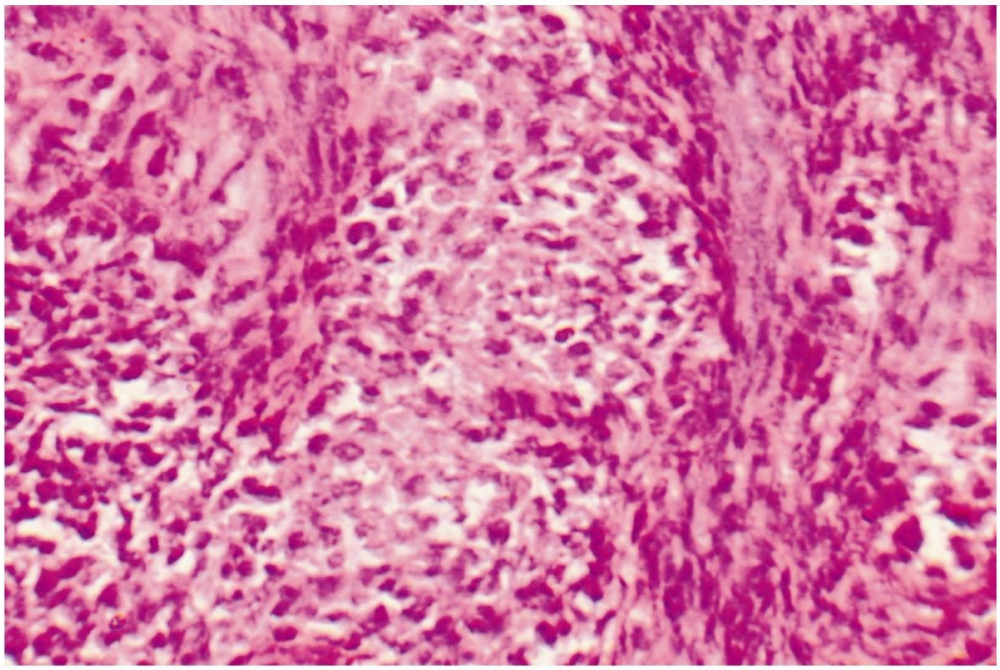


Fig. 18: Desmoplastic Neuroblastoma. Micronodular zones of reduced cellularity (Pale Islands) seen. 200x (H&E)

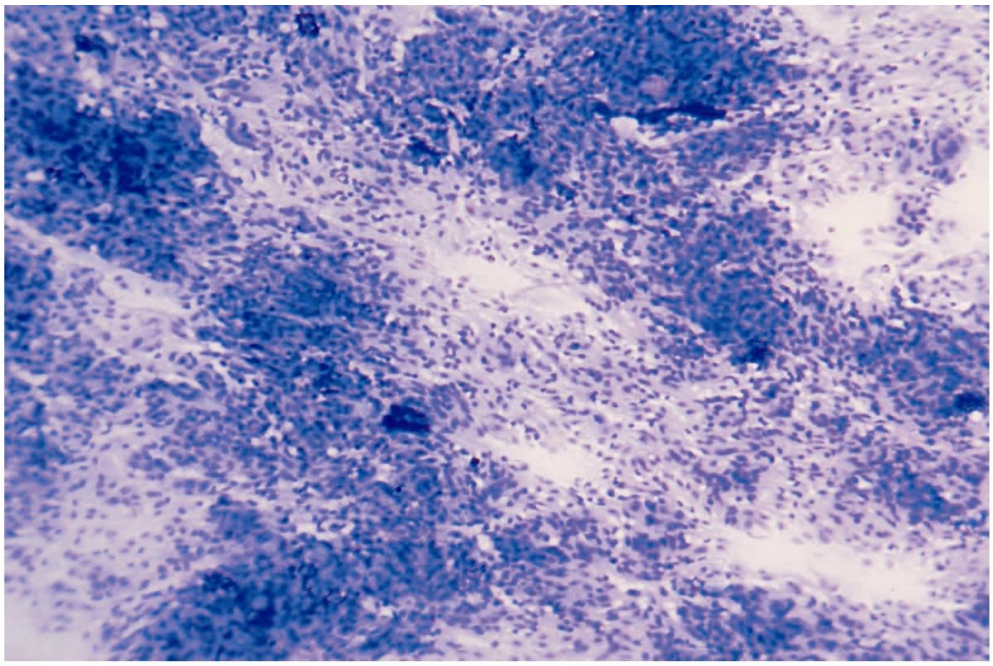


Fig. 19: Meningothelial Meningioma: Smear showing neoplastic cells which are round to oval with indistinct cell borders, eosinophilic cytoplasm and vesicular nucleus disposed off in whorls.50x (Toludine Blue)

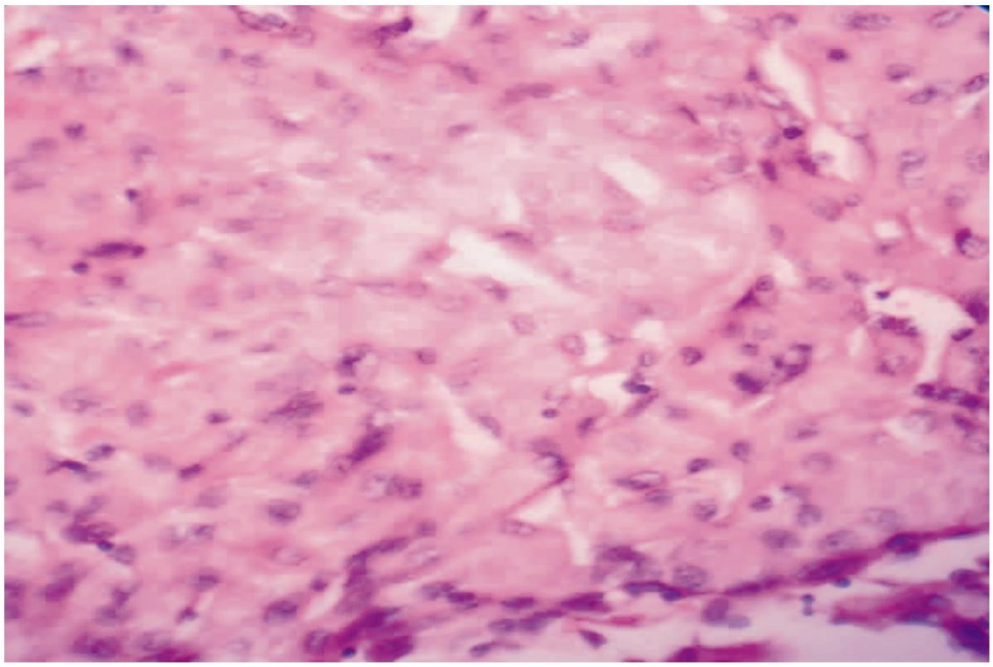


Fig. 20: Meningothelial Meningioma showing meningothelial cells in syncytial arrangement. 200x (H&E)

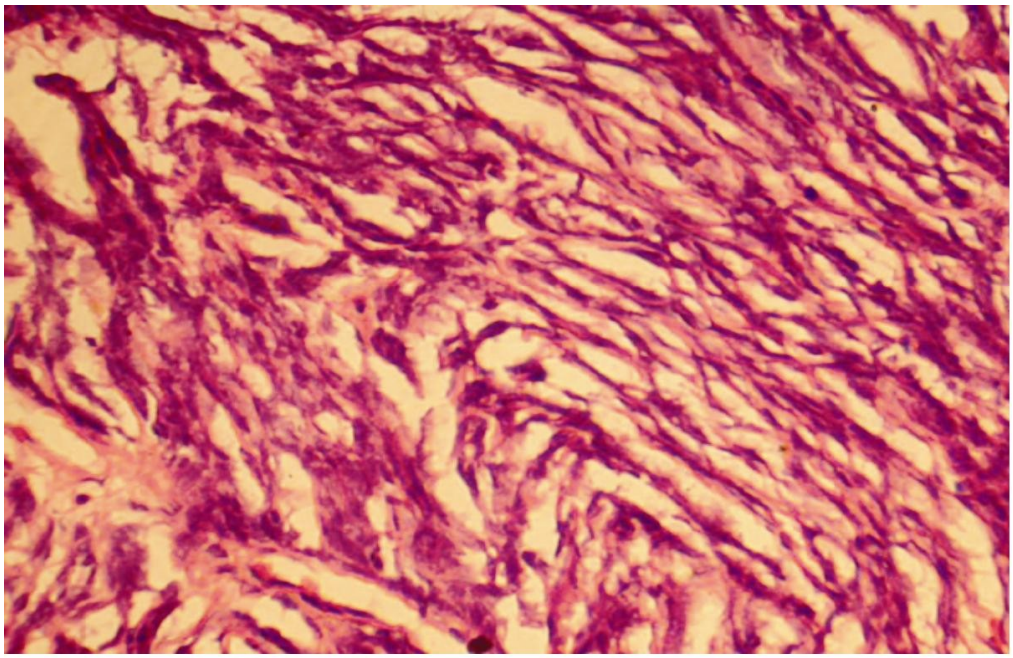


Fig. 21: Fibroblastic Meningioma showing spindle shaped cells arranged in fascicles 200x (H&E)

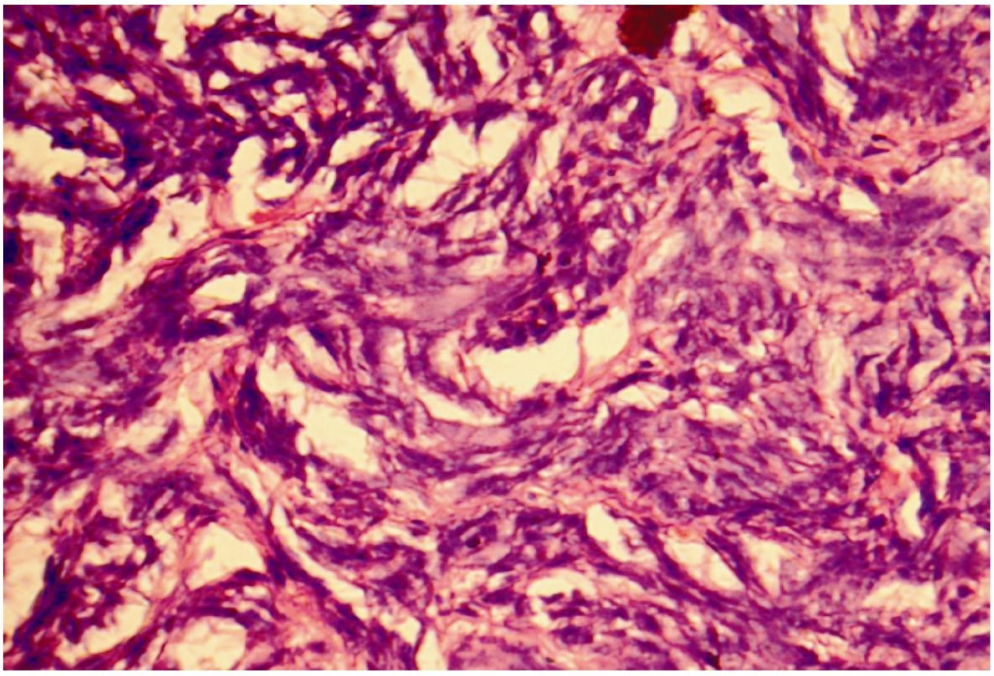


Fig. 22: Fibroblastic Meningioma showing spindle shaped cells arranged in fascicles 200x (H&E)

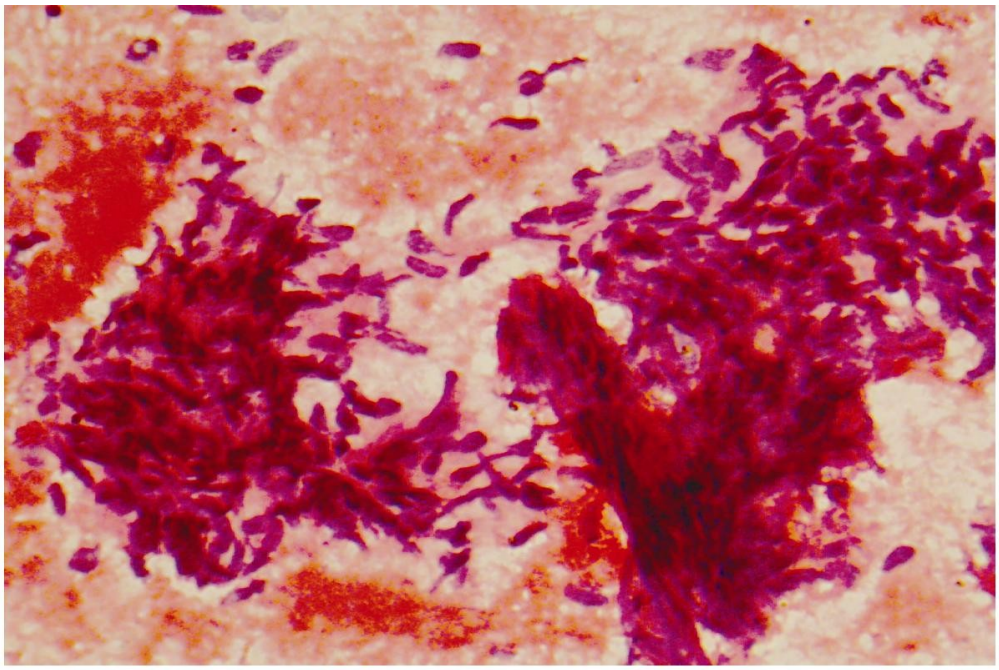


Fig. 23: Schwannoma: smear shows spindle shaped neoplastic cells arranged in discrete groups in an eosinophilic and hemorrhagic background. 200x (H&E)

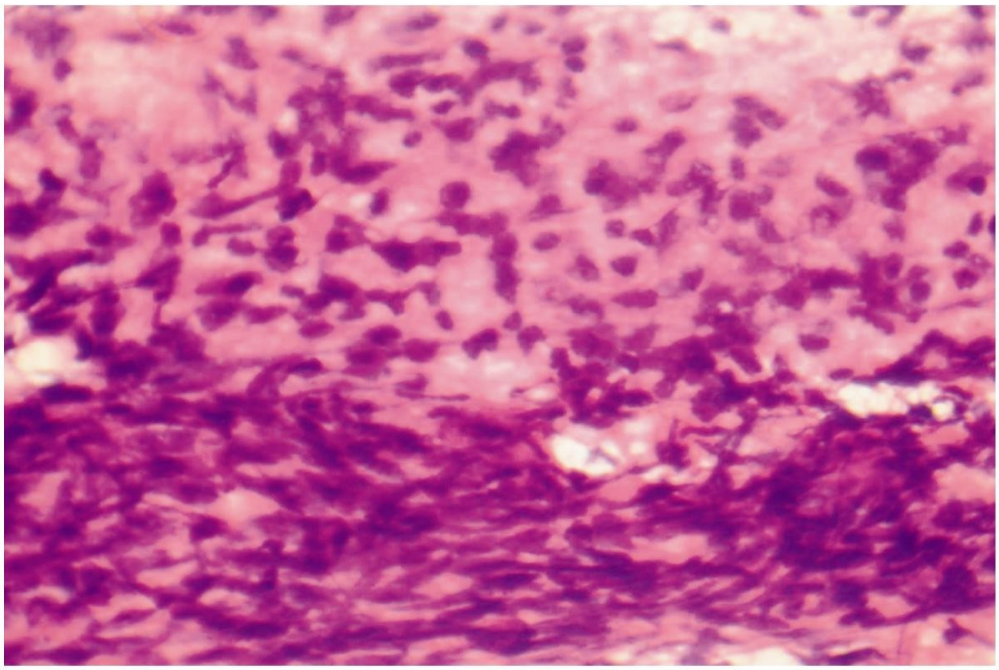


Fig. 24: Schwannoma: smear shows spindle shaped Schwann cells arranged in discrete groups. 200x (H&E)

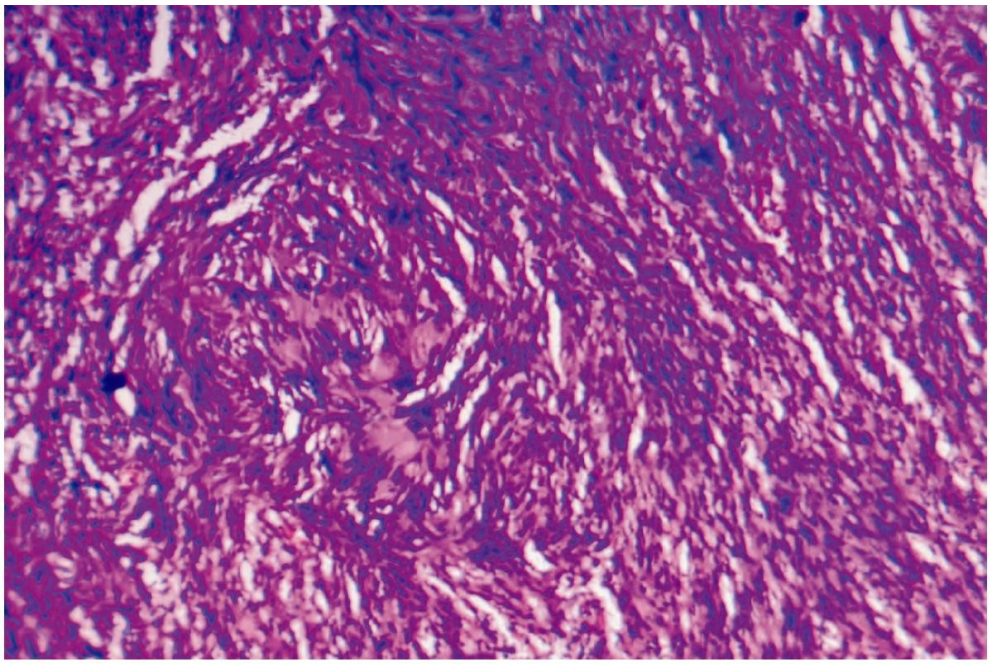


Fig. 25: Schwannoma showing spindle shaped neoplastic Schwann cells with nuclear palisading (Antoni A area with Verocay bodies). Also seen are foamy histiocytes (Antoni B area). 50x (H&E)

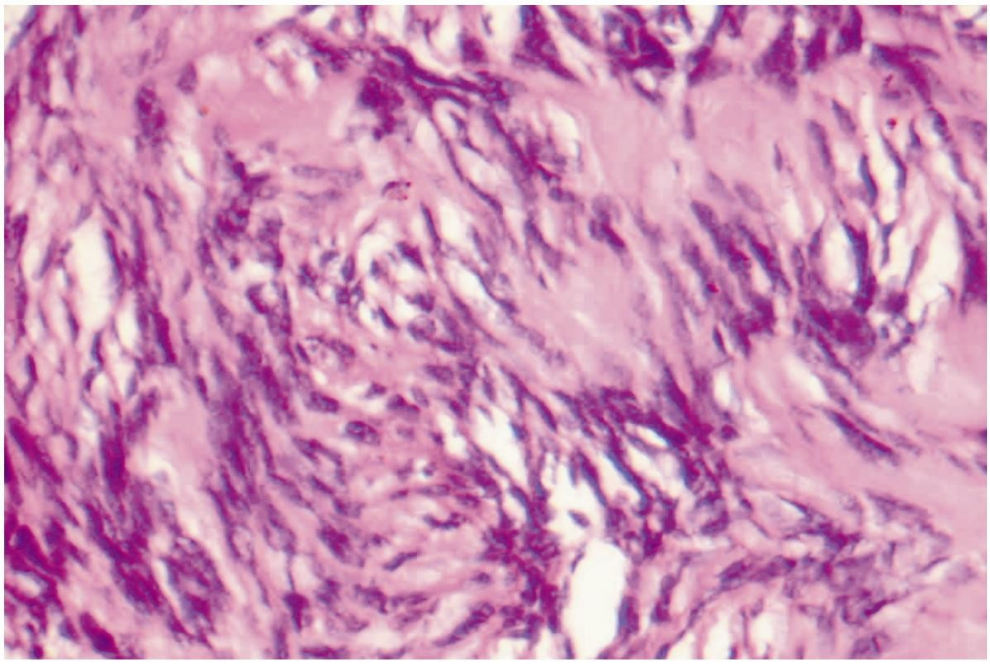


Fig. 26: Schwannoma showing spindle shaped Schwann cells with nuclear palisading (Verocay bodies). 200x (H&E)

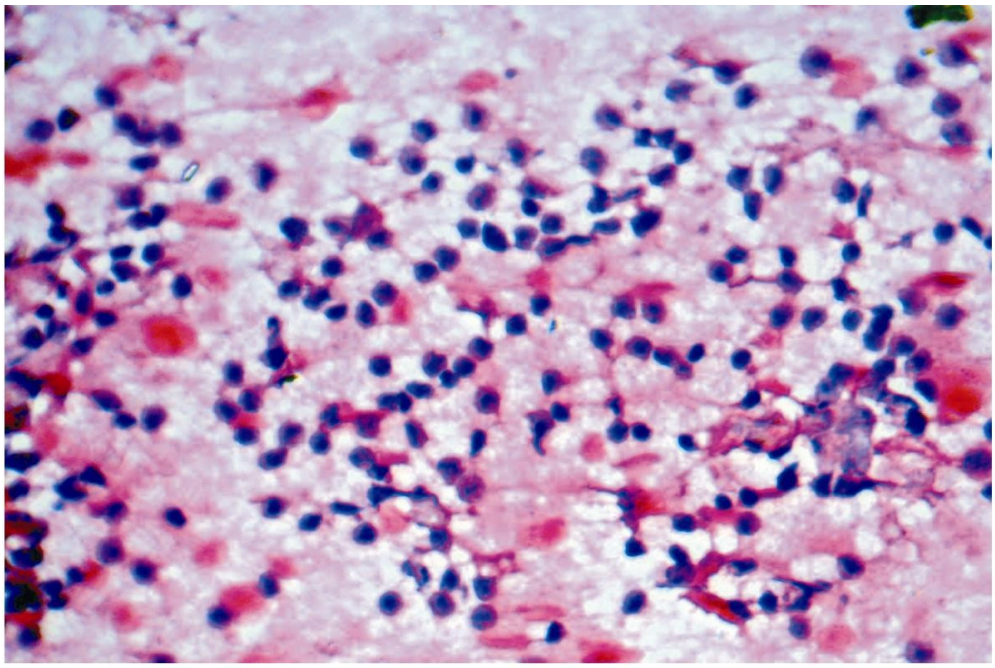


Fig. 27: Pituitary Adenoma. Smear shows cells of varying sizes in an eosinophilic background. 200x (H&E)

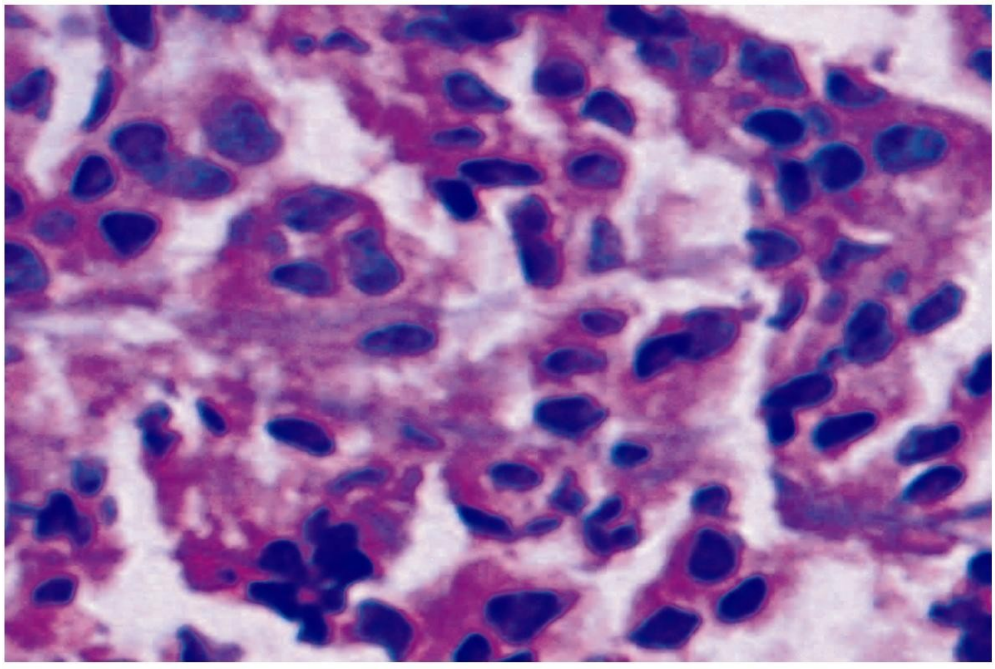


Fig. 28: Pituitary Adenoma showing pleomorphic cells with abundant eosinophilic cytoplasm. Considerable variation in size of the cells seen. 200x (H&E)

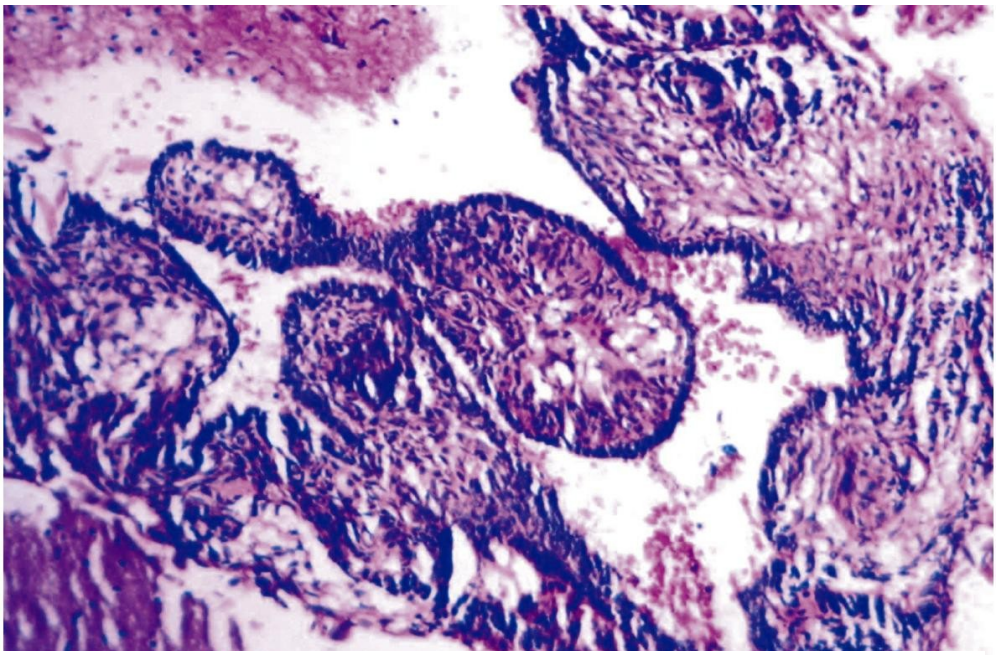


Fig. 29 : Craniopharyngioma showing anastomosing epithelial islands with peripheral palisading of nuclei and a central loose stellate reticulum. 50x (H&E)

palisading of nuclei and a central loose stellate reticulum. 50x (H&E)

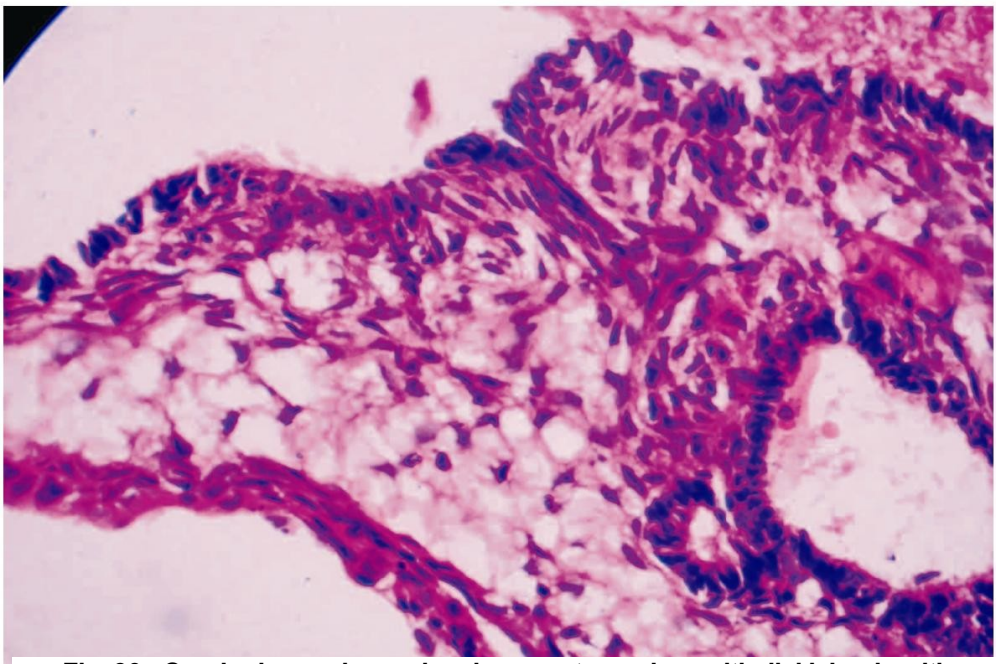


Fig. 30 : Craniopharyngioma showing anastomosing epithelial islands with peripheral palisading of nuclei and a central loose stellate reticulum. 200x (H&E)

palisading of nuclei and a central loose stellate reticulum. 200x (H&E)

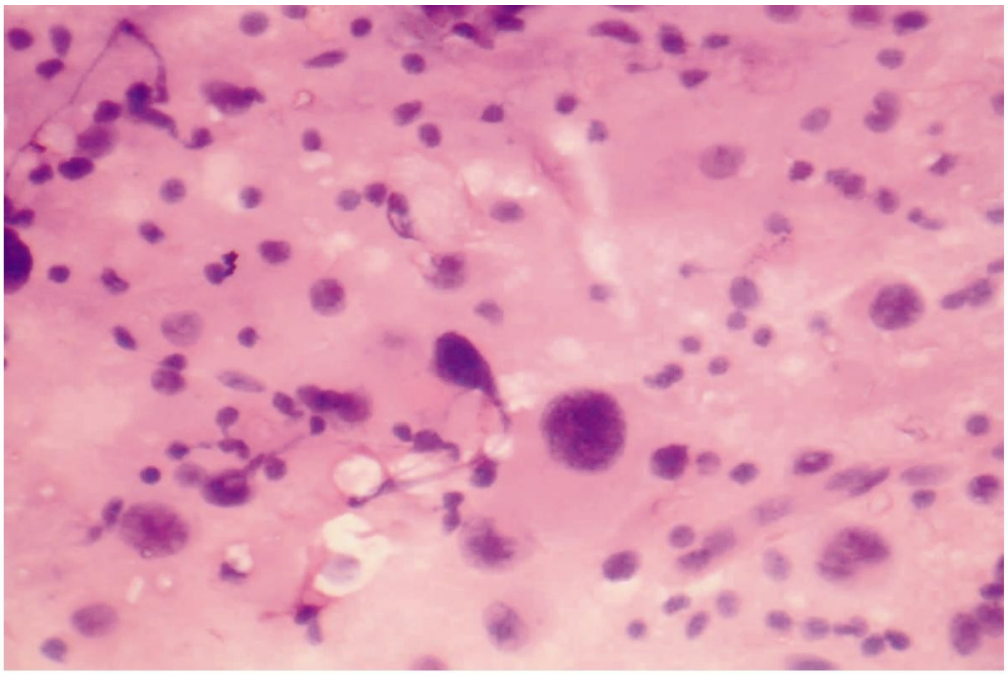


Fig. 31: Metastatic Carcinomatous Deposit. Cellular smear showing tumor cells with eosinophilic cytoplasm and bizarre hyperchromatic nucleus. 200x (H&E)

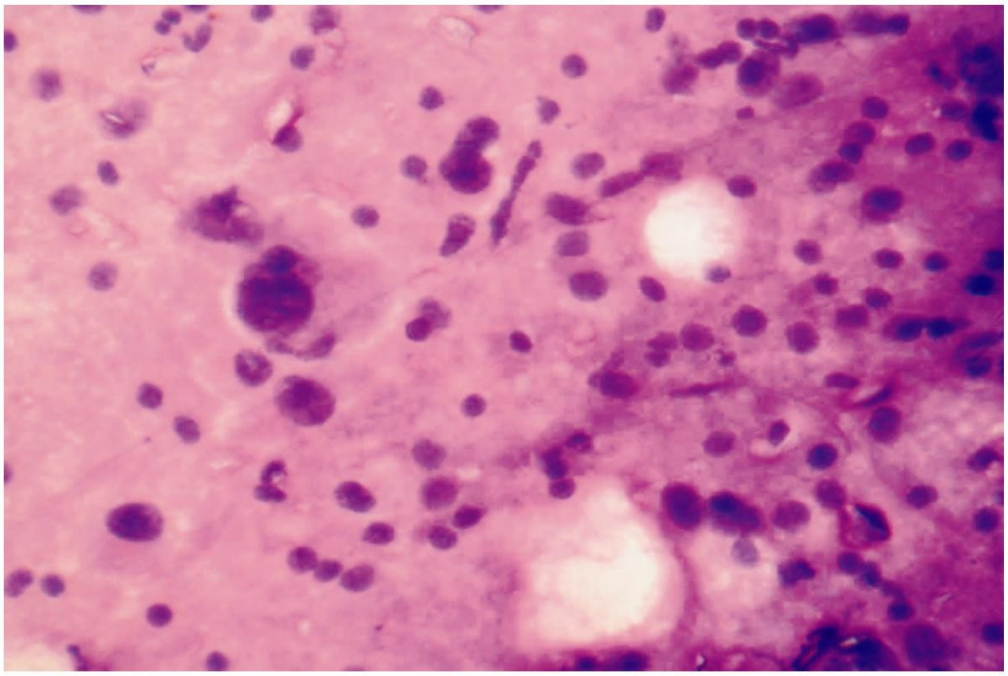


Fig. 32: Metastatic Carcinomatous Deposit. Cellular smear showing tumor cells with eosinophilic cytoplasm and bizarre hyperchromatic nucleus. 200x (H&E)

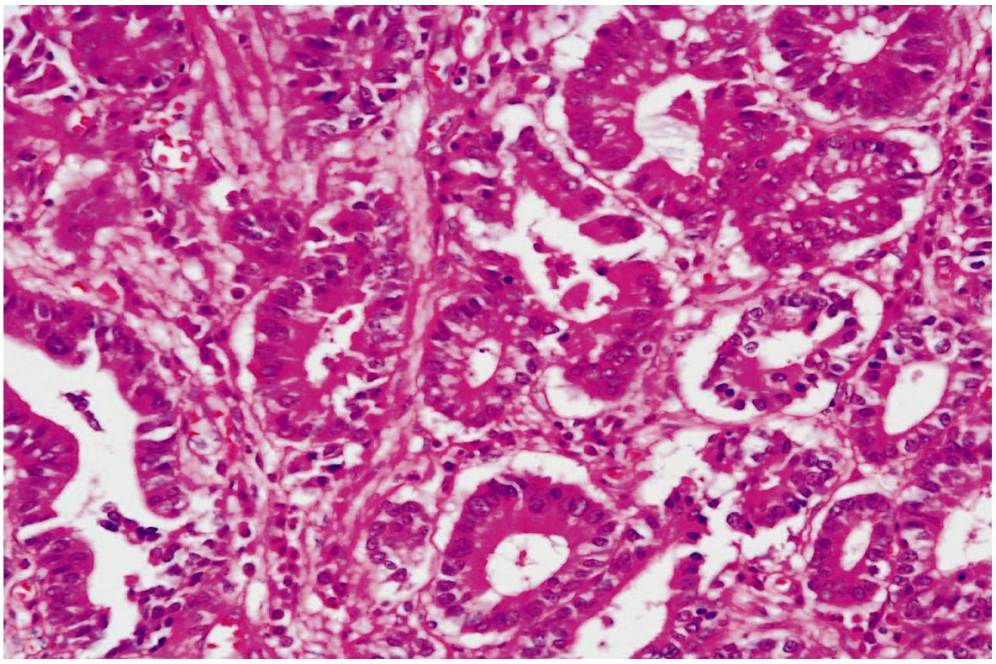


Fig. 33 : Metastatic Carcinomatous Deposit showing tumor cells diposed off in glandular pattern 200 X (H&E)

cytoplasm and bizarre pleomorphic nuclei. The cells appear highly anaplastic. 50x (H&E)

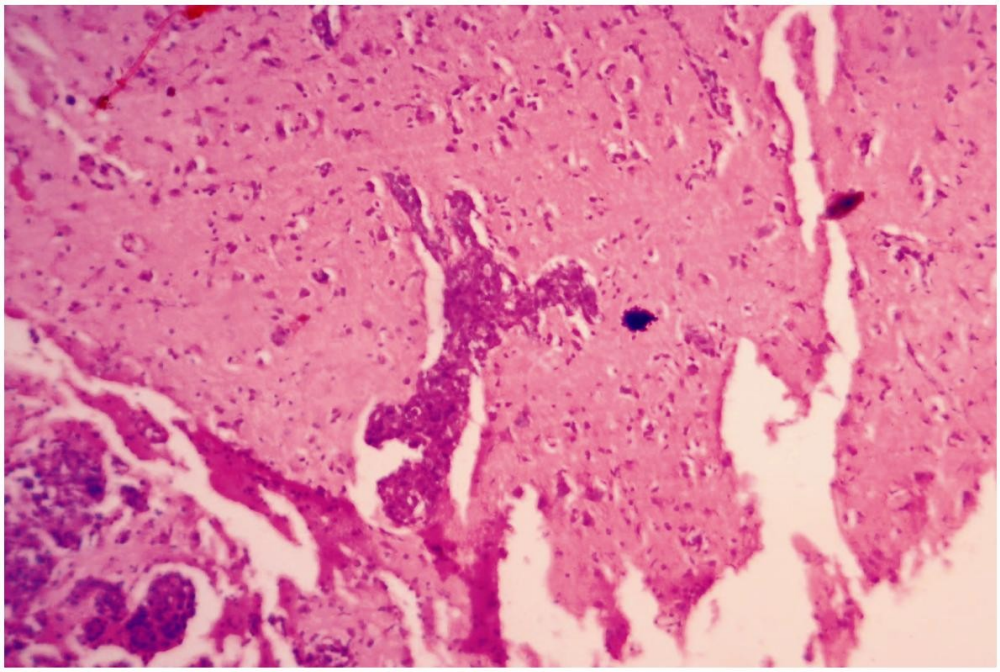


Fig. 34: Metastatic Carcinomatous Deposit showing sheets of tumor cells infiltrating the brain parenchyma. 50x (H&E)

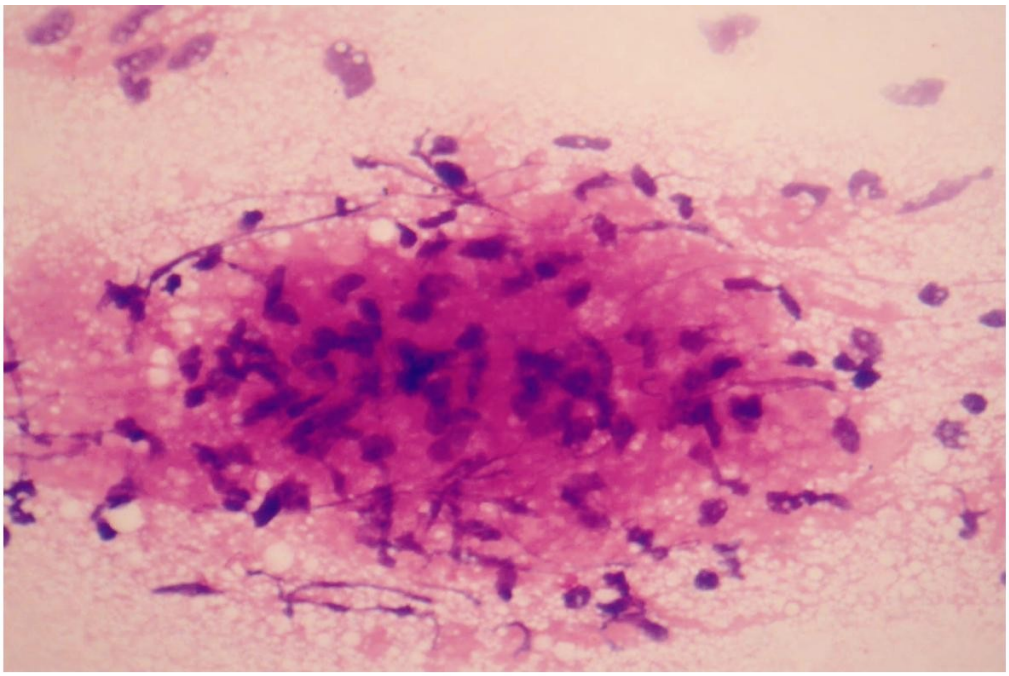


Fig. 35: Tuberculoma showing epitheloid cells and lymphocytes in a necrotic background. 200x (H&E)

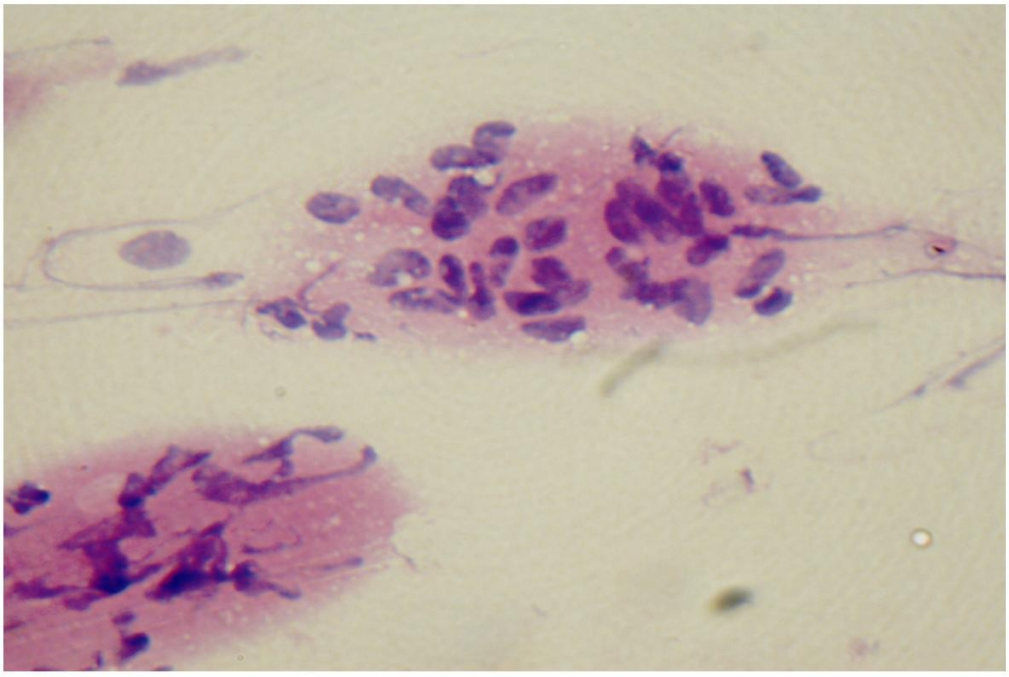


Fig. 36: Tuberculoma showing epitheloid cells and lymphocytes in a necrotic background. 200x (H&E)

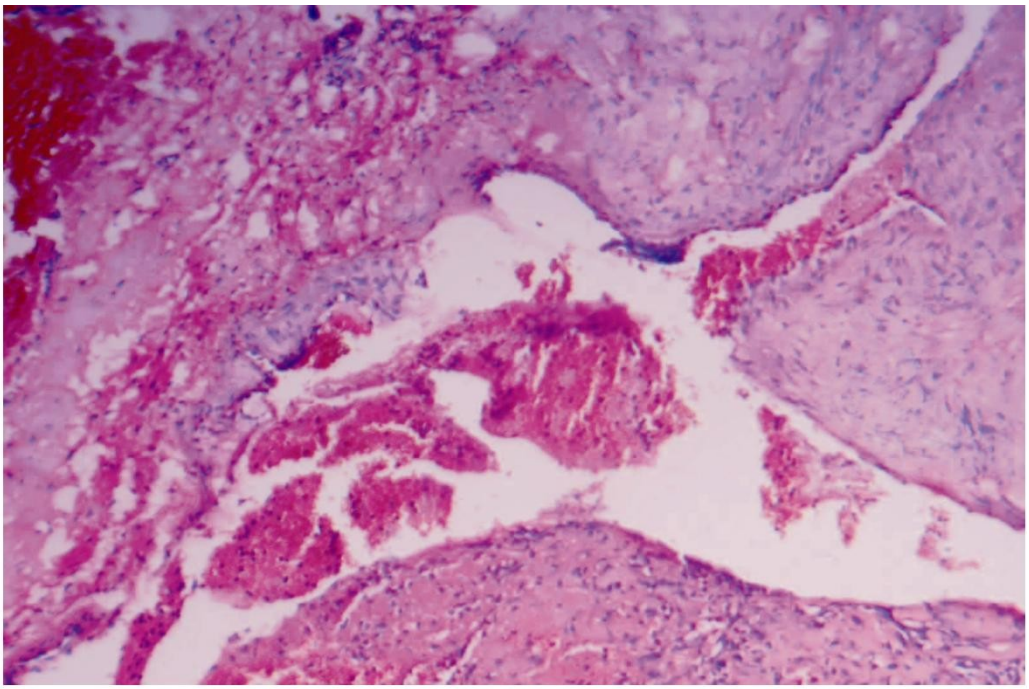


Fig. 37: Lipoma showing mature adipocytes with glial tissue. 50x (H&E)

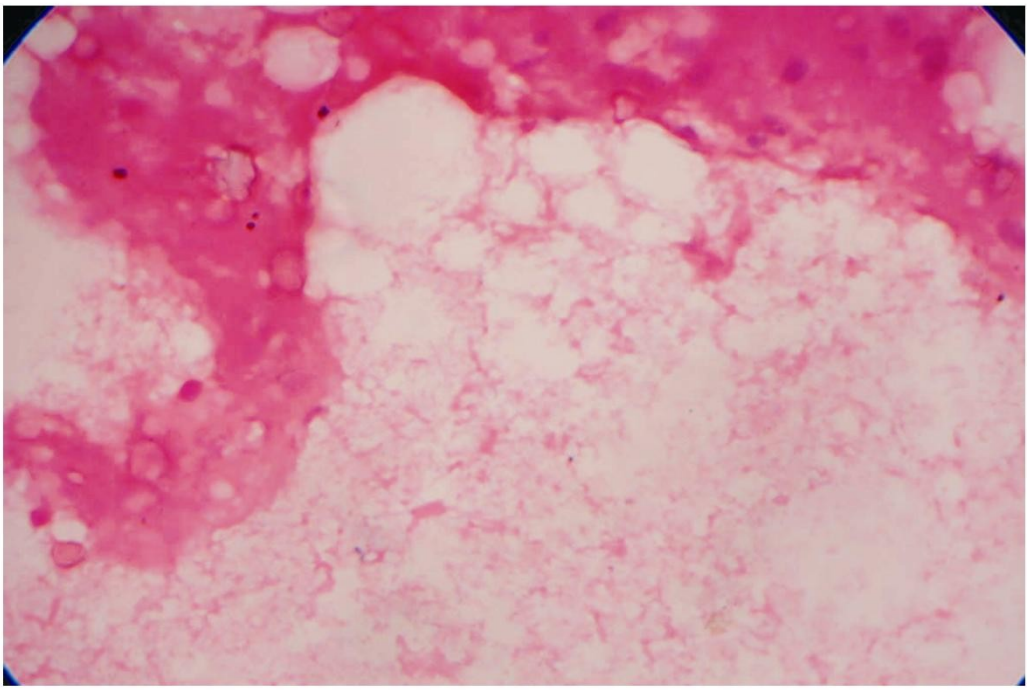


Fig. 38: Cavernoma showing enlarged vessels filled with hemorrhage. 200x (H&E)

Discussion

Discussion

Tumors of the brain have unique characteristics that set them apart from neoplastic processes elsewhere in the body. Even though they amount to less than 2% of all malignant neoplasms, their increased incidence in recent years has created great interest in studying them.

A comparison of the incidence of intracranial space occupying lesions in the present study with that of Dastur and Lalitha study (Dastur et al 1968) and Ramamurthi study (Ramamurthi et al 1973) showed there was an increased incidence of brain tumors in the present study (Table 3).

Table 3: Incidence of Intracranial Space Occupying Lesions – A comparative study

S. No	Type	Dastur & Lalitha Study		Ramamurthi Study		Present Study	
		No. of Cases	% of ICSOL*	No. of Cases	% of ICSOL*	No. of Cases	% of ICSOL*
1	Tumors	1326	80.36	1897	88.93	88	89.79
2	Non-Neoplastic Cyst	21	1.27	25	1.17	2	2.04
3	Tuberculoma	303	18.36	211	9.89	8	8.16

* ICSOL – Intracranial Space Occupying Lesion

Age Incidence

The decennial age incidence of CNS tumors in the present study showed maximum incidence of CNS tumors occurred in the age group of 21-30 years. In the Geetha (Geetha et al 1980) study the maximum incidence of CNS tumors also occurred in the same age group (Table 4). However in a Western study, which was based on biopsy series during the period 1975-1995, at the Institute of Neuropathology, University of Zurich, Switzerland the peak incidence occurred in the 45-70 age group.

Table 4: Decennial Age Incidence of Brain Tumors – A Comparative Study

Decade	Present Study		Geetha Study	
	No of cases	Percentage	No of cases	Percentage
0 – 10	5	5.68	14	8.69
11 – 20	9	10.22	30	18.63
21 – 30	23	26.14	46	28.57
31 – 40	19	21.59	42	26.08
41 – 50	20	22.72	24	14.90
51 – 60	9	10.23	2	1.24
61 – 70	3	3.41	3	1.86

Sex Incidence

A comparison of the sex incidence of CNS tumors with that of Geetha study (Geetha et al 1980) showed that in the present study the male : female ratio was 1.05:1 while in the Geetha study it was 1.9:1 (Table 5).

Table 5: Sex Incidence of Brain Tumors – A comparative study

	Total No. of Cases	Males	Females	Male:Female ratio
Present Study	88	45	43	1.05:1
Geetha Study	161	106	55	1.9:1

The aim of treating Central Nervous System lesions to a large extent depends on the histopathological diagnosis. The main goal of the pathologist is to give a quick and accurate diagnosis to the surgeon as early as possible. Surgery for lesions in the brain is time consuming. The brain is a vital organ and it is highly fragile. Hence repeated surgery is avoided. Opening and closing the skull frequently is also difficult. At the operating table many a times the neurosurgeon wants to know if the tissue taken for biopsy is from a representative site. Hence the pathologist has to bear all this in mind and must be able to arrive at a diagnosis even when the patient is in the operating room. The procedure

adopted to achieve all these parameters must be simple, easy to perform, rapid and accurate. Crush cytology is able to meet most of these requirements.

Frozen sections are now being increasingly replaced by crush preparations. The advantages of crush cytology in comparison with frozen section are that it does not need any expensive and sophisticated instruments like cryostat. The tissue required to give a diagnosis is also very minimal. It does not need an expert to interpret the results. Any trained pathologist will be able to evaluate the lesion fairly accurately over a period of time. Cytological details are also more accurate (Beuvon F et al in 2000).

It takes less than a minute to prepare a good smear. However some lesions are firm and resist spreading. In such cases the tissue has to be made into tiny fragments and then crushed. The most important precaution to be taken after preparing these crushed preparations is to prevent them from excessive drying. If the smears are allowed to dry for a long time, the cells undergo degenerative changes. The cells become swollen and vacuolated with markedly granulated cytoplasm and degenerative changes in the nuclei. In extreme cases the entire cell

became filled with a single vacuole and the cytoplasmic boundaries become disintegrated. This point can be highlighted from the following example

Case 69 left frontal lobe lesion was diagnosed as Malignant Oligodendroglioma on histopathology. It could not be diagnosed by crush preparations because the cells had a lot of degenerative changes due to excessive drying.

Two common artifacts encountered in smears are glove powder and smear artifacts. Widespread smear artifact occurred if too much pressure was applied on tough tissue.. Glove powder may be misinterpreted as psammoma bodies. One should always be on the lookout for any post radiational changes.

In the present study crush cytology was correlated with histopathology. Most of the tumors of neuroepithelial origin like Gliomas were soft and easy to crush and a good smear was obtained.. On the other hand Meningiomas especially Fibroblastic variant, Schwannomas and tumors eliciting desmoplasia like Desmoplastic Neuroblastoma offered considerable resistance entrapping the cells in the fibrous stroma. In the 100 cases studied there was 33 cases of Astrocytoma, 15 cases of

Meningioma, 16 cases of Schwannoma, 3 cases of Mixed Glioma, 2 cases of Oligodendroglioma, 3 cases of Medulloblastoma, 2 cases of Ependymoma, 1 case of Desmoplastic Neuroblastoma, 1 case of Primitive Neuroectodermal Tumor, 2 cases of Metastatic Carcinomatous Deposit, 3 cases of Pituitary Adenoma, 1 case of Craniopharyngiomas, 1 case of Hemangiopericytoma, 1 case of Vascular Neurofibroma, 2 cases of Cavernoma, 1 case of AV Malformation, 8 cases of Tuberculoma, 2 cases of Epidermal Cyst, 2 case of Cerebral Abscess, 1 case of Lipoma.

Roesslers had done retrospective analysis of 4172 patients operated from 1985-1999. There was complete correlation with the final diagnosis in 89.8% of cases. Diagnostic accuracy increased to 95% when cases of partial correlation, mainly due to grading deviations were included. In Firlik KS study 595 cases were retrospectively reviewed. Intraoperative Cytological diagnosis correlated with the final diagnosis in 92% of cases. (54% complete correlation and 38% partial correlation). In the Present Study Out of 100cases analysed Complete correlation with the final diagnosis was achieved in 82% of cases. Diagnostic accuracy increased to 92% when cases of partial correlation mainly due to grading deviations were included (Table 6).

Table 6: Comparative Correlation Between Crush Cytology & Histopathology – A comparative study

Name of the study	Correct Diagnosis in %	Complete Correlation in %	Partial Correlation in %
Firlik KS 1999	90	52	38
Roessler K 2002	95	89.8	5.2
Present Study	90	80	10

The Sensitivity and Specificity of Neuroepithelial Tumors were 86.67% and 98.18% respectively. The Positive predictive value was 97.5% and the Negative predictive value was 90% (Table 7).

Table 7: Sensitivity and Specificity of Neuroepithelial Tumors

		Histopathology Detection		
		Disease Present	Disease Absent	Total
		45	55	
Crush Cytology Detection	40	39 a	1 b	40 (a+b)
	60	6 c	54 d	60 (c+d)
		45 (a+c)	55 (b+d)	

Sensitivity = (a * 100)/(a+c) = 86.67%

Specificity = (d * 100) / (b + d) = 98.18%

Percentage of False Positive = (b * 100) / (b + d) = 0.02%

Percentage of False Negative = (c * 100) / (a + c) = 13.3%

Positive Predictive Value = (a * 100) / (a + b) = 97.5%

Negative Predictive Value = (d * 100) / (c + d) = 90%

In the present study there were 4 cases of missed diagnosis due to improper smear technique. Case 79 a case of Malignant Oligodendroglioma was missed because of drying artifact and degenerative changes in the smear. Case No. 28 a case of Schwannoma the tissue was firm and resisted spreading to form a smear. Hence the material obtained was inadequate for definitive opinion. The material was too necrotic to give any conclusive opinion on 2 cases of Mixed Glioma and Anaplastic Astrocytoma.

10 cases showed only partial correlation with the final diagnosis. 3 of these cases were Astrocytoma whose grading was incorrect. Similarly in 4 cases of Meningioma the final histological typing varied. Case No. 91 a case of Oligoastrocytoma was diagnosed as Oligodendroglioma in crush preparation. This was because of improper sampling technique. The tissue crushed had only Oligodendroglial component. Hence the astrocytic component was missed. This proves that grading of Gliomas

by Daumas – Duport and WHO methods are not of much help in crush preparations. The sample of the tumor may not be characteristic of the whole lesion and some times the grading was a level lower or higher than the final histopathological diagnosis. William H. Mc. Menemy (1960) points out that Kernohan is essentially a practical grading system devised for surgeons and that it is not meant to be a scientific classification of Gliomas. The principle of grading has also been condemned by Russell and Rubinstein who opined that accurate grading can be done only when the entire tumor is available. This may be possible most of the times only at necropsy. Similarly histological variants of a tumor may also be difficult to interpret in crush cytology.

33 cases of Astrocytomas were diagnosed by crush cytology. One important finding in all Astrocytomas were that the cells were attached to their vessel walls by their long glial fibers and food processes. Asha et al (1989) and Xi Hua Yue et al (1987) have emphasized this.

Most of the Astrocytomas showed uniform cell type with pale eosinophilic cytoplasm and round to slightly oval nucleus and delicate glial fibers. The fibers were thin and formed a network in which the cells were enmeshed. In the Gemistocytic type of Astrocytoma the cells

showed oval brightly eosinophilic cytoplasm with a peripherally placed nucleus. These could be easily identified in the smears. In the Pilocytic type of Astrocytomas which were in the posterior fossa eosinophilic globular or fibrillary masses which were cylindrical or coiled were seen. These were Rosenthal fibers.

Case 24 – a case of Astrocytoma Grade IV was diagnosed as lymphoma on crush preparation because the round lymphoid cells were admixed with reactive astrocytes causing interpretational difficulties. (Shanop1999 et al). Case 73 – a case of Pilocytic Astrocytoma was diagnosed as Meningioma on crush preparation due to the presence of calcification and small cells with indistinct cytoplasm. No definitive pattern was identified on the smear. Case 82 – a case of Astrocytoma Grade IV was diagnosed as Sub Ependymoma on crush preparation. This was because of the dense fibrillary background which is encountered in cases of Sub Ependymoma. In Ependymoma also the tumor cells attach to blood vessels causing interpretation difficulties from Astrocytoma.(Adekumle 2005 et al).

The next large group of tumors comprised of Schwannoma. There were 16 cases of Schwannoma. Most of them were present in the

cerebellopontine angle. Schwannoma was also difficult to squash and prepare a good smear. Most of the Schwannoma were seen in females. The presence of plump spindle shaped cells, variation in size of the cells and nuclei, occasional pleomorphic hyperchromatic nuclei and admixture of foamy histiocytes and mononuclear cells helped in the diagnosis. Under low power the crush tissue presented as thick irregular fragments with sharp borders. The palisading pattern of tumor cells were rarely observed. Nguyen G.K. Johnson ES et al (1988) have provided a table for differentiating Meningiomas and Schwannoma

	Meningioma	Neurilemomas
Tumor tissue consistency	Easy to crush	Difficult to crush
Smear Pattern at low power	Delicate sheets and clusters of tumor cells	Irregular large and thick fragments of tumor tissue
Crushing Artifacts	Almost absent	Marked
Cellular features Nucleus Cytoplasm and cell	 Regular round or oval Thin fairly well defined, variable in size, polygonal or spindle shaped depending on	 Pleomorphic hyperchromatic or pyknotic Ill-defined or variable

	tumor sub type	
Other Features	Psammoma bodies may be present, foamy histiocytes rarely seen	No Psammoma bodies, foamy histiocytes commonly present

There were 15 cases of Meningiomas. Nine patients were above 40 years. 10 cases were Meningothelial Meningioma. There were 3 cases of fibroblastic Meningioma. Because of the fibrous component it was difficult to crush the tissue in these cases and produce a good smear. Case 86 – a case of Mixed Glioma with predominantly astrocytic component was diagnosed as Fibroblastic Meningioma on crush preparation. Difficulties to differentiate a Fibroblastic Meningioma from Astrocytoma can occur because of the fibrillary background seen in Astrocytoma. According to Stephen Silverberg when these kind of interpretational difficulties are encountered, some focal areas in Fibroblastic Meningioma still would show the presence of whorls and arachnoidal cells which would help to clinch the diagnosis. The cytoplasm of Meningioma cells may superficially mimic cell processes of neoplastic astrocytes but Meningioma cells appear soft looking and are more delicate. (Shanop 1999 et al).

There was one case of Psammomatous Meningioma. It is easy to diagnose this on crush preparation because of the large number of calcific bodies. The grittiness while smearing also gave a clue. But calcific bodies can also be seen in Oligodendroglioma and Pilocytic Astrocytoma. Hence other features of Meningioma must be looked into when one encounters a smear with calcific bodies. Case 77 a 42 year old male was diagnosed as Atypical Meningioma on crush preparation. But the histopathology diagnosis turned out to be Meningothelial Meningioma. Cells at the periphery of a smear may artifactually appear bigger and mimic Atypical Meningioma. Hence the typing of Meningiomas is not precise on smears. Similarly a 40 year old female was diagnosed to have Meningioma Angiomatous type on crush cytology. But the histopathology turned out to be Hemangiopericytoma. Hence crush cytology has its limitations and the entire architectural pattern seen in biopsy cannot be appreciated in smears.

3 cases of Medulloblastoma were diagnosed. 1 case was in the posterior fossa. The other 2 cases were drop metastasis in spine from cerebellum. All the cases were seen in the younger age group. All the 3 cases were diagnosed accurately on crush preparation. Medulloblastoma however at times is difficult to differentiate from Oat

cell carcinoma of the lung and peripheral Neuroblastoma metastatising to the central nervous system. Case No 55 was diagnosed as Desmoplastic Neuroblastoma. In smear preparations and even in histopathology it is difficult to differentiate Desmoplastic Neuroblastoma from Desmoplastic Medulloblastoma. This tumor was located in the frontal region. This enabled us to swing the pendulum in favor of Desmoplastic Neuroblastoma. Hence it is mandatory to look into the clinical history and CT findings of the patient before arriving at a diagnosis.

2 cases of Ependymoma were diagnosed. 1 case was in the pediatric age group and the lesion was present in the lateral ventricle. The other case was a 30 year old man with a D12-L2 lesion. Both the cases were diagnosed accurately on crush preparation. In Ependymoma the cells were closely attached to the vessels producing perivascular pseudo rosettes. A highly papillary pattern was seen in the smear of the 30 year old man. An obvious stroma and several layers of tumor cells were seen. The cells were columnar in appearance. The background had a myxoid appearance. Histopathological diagnosis of this case was Myxopapillary type of Ependymoma.

3 cases of pituitary adenoma were diagnosed both on smear and histopathology. The tissue was soft and smeared out easily. Sheets of oval and round cells were seen. They were pleomorphic with varying amounts of pale cytoplasm and conspicuous nucleoli. Binucleate and multinucleate cells were also seen. A papillary pattern was also seen in one of the smears. A total lack of glial background helped to distinguish it from an Ependymoma. Alex Landolt and Hugo Krayenbuhl in 1972 modified the cytological technique for rapid differentiation of pituitary adenomas. The toludine blue had been modified by the addition of orange G as cytoplasmic stain. This method allowed the differentiation of somatotrophic, lactotrophic and endocrine inactive tumors.

There was 1 case of Craniopharyngiomas. The patient was in the younger age group. The lesion was located in the suprasellar region. On squashing it did not smear well, appeared membranous and revealed basaloid cells topped by squamous cells. There was no mitosis or multinucleate cells.

Two cases of Metastatic carcinoma were diagnosed. 1 case was diagnosed as Astrocytoma Grade IV on crush preparation. In Metastatic carcinoma the tumor cells tend to separate from the vessel wall. The

cells spread out evenly and individually with the result the cells had well defined outlines. This tendency of the tumor cells to separate from the vessel wall clearly distinguished it from Astrocytoma.

There was one case of Vascular Neurofibroma diagnosed by crush and confirmed by histopathology. The cells were characteristically spindle shaped and the background had a myxoid matrix. One case of Lipoma, 2 case of Cerebral Abscess and two cases of Cavernous Angioma diagnosed on crush cytology correlated accurately with histopathology.

Epidermoid cyst could be easily identified by the presence of numerous anucleate squames in a granular eosinophilic background. There were 2 such cases, correlated well with histopathology.

There were 7 cases of Tuberculomas diagnosed on crush cytology and histopathologically proven. All the 7 cases had epitheloid cell collection in a necrotic background. Case No. 76 was diagnosed as Tuberculoma on crush preparation, CT also confirmed the diagnosis but when the remaining tissue was processed for paraffin section it showed only normal brain tissue. The abnormal tissue might have been utilized for smear preparation. In such instances crush cytology is complementary to histopathology in arriving at a diagnosis.

As there were no cases of Hemangioblastoma, Myeloma, Chordoma, Pineal tumors and Tumors of the Choroid Plexus the efficiency of squash preparation could not be commented in these cases. However other authors (Shanop 1999 et al and Stephen et al 2001) have exemplified the usefulness of squash preparation in these tumors also.

Summary and Conclusion

Summary and Conclusion

100 cases during a period of 2 years from January 2004 to January 2006 of Space Occupying Lesions of Central Nervous System were studied using squash technique. The cytodiagnosis and histopathology diagnosis were correlated.

Out of 100 space occupying lesions 88% were tumors, 8% Tuberculomas, 2% non neoplastic cysts and 2% Abscess.

The peak age incidence of brain tumors was seen in the age group between 21- 30 years.

45 were males and 43 were females.

Cytopathology of central nervous system in squash preparation with histological correlation was done in 100 cases..

Of the total 100 cases, complete correlation with the final diagnosis was achieved in 82% of cases. Diagnostic accuracy was increased to 92% when cases of partial correlation mainly due to grading deviations were included. The sensitivity and specificity of Neuroepithelial Tumors were

86.67% and 98.18% respectively. The Positive predictive value was 97.5% and the Negative predictive value was 90%.

10% partial correlation defect was not significant enough to affect the management of the patient.

Correlation with CT and clinical details were helpful in improving the accuracy rate.

The problems encountered were

1. Improper squash preparation
2. Sampling error leading to grading discrepancy
3. Sampling error leading to inadequate diagnosis in cases of mixed lesions

In conclusion, it was possible to make a correct diagnosis by squash preparation in 92% of the cases.

Crush cytology is a valuable, simple, easily reproducible, cost effective and clinically significant procedure. It could be used as an intraoperative diagnostic tool to assist the surgeon for preoperative treatment plan.

Master Chart

Master Chart

(January 2004 to January 2006)

S.#	Patient Name	Age	Sex	Site	CT Diagnosis	Crush Diagnosis	Histopath Diagnosis
1	Fatima	19	F	Cerebellar	Tuberculoma	Tuberculoma	Tuberculoma
2	Ramasamy	60	M	Temporal	Lipoma	Lipoma	Lipoma
3	Alamelu	45	F	Spinal L2	Schwannoma	Schwannoma	Schwannoma
4	Dhanalakshmi	42	F	Spinal L2	? Schwannoma ?Neurofibroma	Schwannoma	Schwannoma
5	Jayakumar	10	M	Spinal C2-C6	Medulloblastoma	Medulloblastoma	Medulloblastoma
6	Indrani	65	F	Spinal C2-C4	Meningioma	Meningothelial Meningioma	Meningothelial Meningioma
7	Thangavel	48	M	Parietal	Choroid Plexus Papilloma	Glioblastoma Multiforme	Glioblastoma Multiforme
8	Subashree	13	F	Cerebellar	Medulloblastoma	Medulloblastoma	Medulloblastoma
9	Gangammal	37	F	Parietal	Meningioma	Glioblastoma Multiforme	Glioblastoma Multiforme
10	Amudha	40	F	Cerebello Pontine Angle	Schwannoma	Schwannoma	Schwannoma
11	Narmada	5	F	Frontal	PNET	PNET	PNET
12	Vivek kumar	20	M	Temporal	Epidermal cyst	Epidermal Cyst	Epidermal Cyst
13	Mahesh	21	M	Lateral Ventricle	Meningioma	Astrocytoma Grade2	Astrocytoma Grade2
14	Durgadevi	25	F	Frontal	Glioma	Necrotic material	Oligoastrocytoma
15	Maryammal	56	F	Spinal D5	Meningioma	Psammomatous Meningioma	Psammomatous Meningioma
16	Ramalingam	49	M	Orbital	Meningioma	Meningothelial Meningioma	Fibroblastic Meningioma
17	Pramis	42	M	Frontal	Glioma	Astrocytoma Grade2	Astrocytoma Grade2
18	Seetharaman	47	M	Cerebello Pontine Angle	Schwannoma	Schwannoma	Schwannoma
19	Kanniapan	70	M	Temporal	Glioblastoma	Necrotic material	Anaplastic Astrocytoma
20	Chandrasekhar	41	M	Frontal	Glioblastoma	Glioblastoma Multiforme	Glioblastoma Multiforme
21	Malliga	30	F	Frontal	Abscess	Abscess	Abscess
22	Manoj	42	M	Frontal		Anaplastic Astrocytoma	Anaplastic Astrocytoma
23	Venkatesan	25	M	Cerebello Pontine Angle	Schwannoma	Schwannoma	Schwannoma
24	Rathinammal	50	M	Occipital	Glioma	Lymphoma	Glioblastoma Multiforme
25	Vairasunda	40	M	Spinal D11-L1	AV	Cavernoma	Cavernoma

	ram				Malformation		
26	Rani	38	F	Temporal	Schwannoma	Schwannoma	Schwannoma
27	Elumalai	40	M	Temporal	Schwannoma	Astrocytoma Grade2	Astrocytoma Grade2
28	Ismail	3	M	Optic Nerve	Meningioma	Material scanty	Schwannoma
29	Rajagopal	40	M	Frontal	Tuberculoma	Tuberculoma	Tuberculoma
30	Thaiyal nayagi	35	F	Cerebellar	Cystic Astrocytoma	Diffuse Astrocytoma	Diffuse Astrocytoma
31	Murugan	27	M	Cerebellar	Pilocytic Astrocytoma	Astrocytoma Grade 2	Astrocytoma Grade 2
32	Saheera Banu	25	F	Optic Nerve	Schwannoma	Schwannoma	Schwannoma
33	Jancy Mary	35	F	Cerebellar	Meningioma	Meningothelial Meningioma	Meningothelial Meningioma
34	Balaji	23	M	Cerebellar	Glioma	Astrocytoma Grade 2	Astrocytoma Grade 2
35	Vijaya Kumar	21	M	Cerebellar	Abscess	Abscess	Abscess
36	Kulzar Begum	43	F	Temporal	Schwannoma	Schwannoma	Schwannoma
37	Gopalakrishnan	40	M	Parietal	Glioma	Astrocytoma Grade 2	Astrocytoma Grade 2
38	Laxmi	35	F	Cerebello Pontine Angle	Meningioma	Meningothelial Meningioma	Angiomatous Meningioma
39	Sivakumar	25	M	Lateral Ventricle	Glioma	Anaplastic Astrocytoma	Anaplastic Astrocytoma
40	sublaxmi	13	F	Temporal	Glioma	Anaplastic Astrocytoma	Anaplastic Astrocytoma
41	Ellapan	52	M	Frontal	Secondaries	Metastatic Carcinomatous Deposit	Metastatic Carcinomatous Deposit
42	Mohan	24	M	Sphenoidal	Meningioma	Meningothelial Meningioma	Meningothelial Meningioma
43	Jayakumar	24	M	Para Sagittal	Glioma	Anaplastic Astrocytoma	Anaplastic Astrocytoma
44	Mohana	35	F	Temporal	Meningioma	Meningothelial Meningioma	Fibroblastic Meningioma
45	Punitha	43	F	Sellar	Pituitary Adenoma	Pituitary Adenoma	Pituitary Adenoma
46	Ravanammal	39	F	Parietal	Glioma	Anaplastic Astrocytoma	Anaplastic Astrocytoma
47	Ramamoorthy	26	M	Temporal	Schwannoma	Epidermal Cyst	Epidermal Cyst
48	Umapathy	60	M	Parietal	Glioma	Anaplastic Astrocytoma	Anaplastic Astrocytoma
49	Arumugam	40	M	Olfactory Groove Anterior	Meningioma	Meningothelial Meningioma	Meningothelial Meningioma
50	Raj	42	M	Frontal	Tuberculoma	Tuberculoma	Tuberculoma
51	Vasantha	19	F	Parietal	Glioma	Astrocytoma Grade 2	Astrocytoma Grade 2
52	Kala	33	F	Temporal	Glioma	Astrocytoma Grade 2	Astrocytoma Grade 2
53	Lavanya	22	F	Cerebello	Schwannoma	Schwannoma	Schwannoma

				Pontine Angle			
54	Rasool Banu	22	F	Cerebello Pontine Angle	Schwannoma	Schwannoma	Schwannoma
55	Raja	7	M	Frontal	PNET	PNET	Desmoplastic Neuroblastoma
56	Arul Saravanan	33	M	Sellar	Pituitary Adenoma	Pituitary Adenoma	Pituitary Adenoma
57	Raghavan	25	M	Frontal	Tuberculoma	Tuberculoma	Tuberculoma
58	Gajalakshmi	51	F	Spinal C4-T1	Schwannoma	Schwannoma	Schwannoma
59	Baba	45	M	Spinal C7-D1	Neurofibroma	Vascular Neurofibroma	Vascular Neurofibroma
60	Amaravathi	29	F	Frontal	Meningioma	Meningothelial Meningioma	Meningothelial Meningioma
61	Usha	17	F	Spinal D2-D3	Meningioma	Meningothelial Meningioma	Meningothelial Meningioma
62	Srinivasan	41	M	Orbital	Meningioma	Fibroblastic Meningioma	Fibroblastic Meningioma
63	Logeshwari	22	F	Parietal	Cavernoma	Cavernoma	AV Malformation
64	Karthik	28	M	Frontal	Glioma	Astrocytoma Grade 2	Astrocytoma Grade 2
65	Gandhi Raj	35	M	Spinal C6-D3	Glioma	Fibrillary Astrocytoma	Fibrillary Astrocytoma
66	Srividhya	7	F	Lateral Ventricle	Ependymoma	Ependymoma	Ependymoma
67	Maheshwaran	25	M	Frontal	Glioma	Astrocytoma Grade 2	Astrocytoma Grade 2
68	Chandra	40	F	Intra Ventricular	Meningioma	Angiomatous Meningioma	Hemangiopericytoma
69	Balaji	30	M	Spinal D12-L2	Ependymoma	Ependymoma	Ependymoma
70	Kaleeswari	25	F	Parietal	Glioma	Astrocytoma grade 4	Astrocytoma grade 4
71	Robert Antony	17	M	Cerebellar	Glioma	Pilocytic Astrocytoma	Astrocytoma Grade 2
72	Perumal	48	M	Cerebello Pontine Angle	Schwannoma	Schwannoma	Schwannoma
73	Deepa	15	F	Parietal	Choroid Plexus Papilloma	Fibroblastic Meningioma	Pilocytic Astrocytoma
74	Usha	43	F	Spinal C3-C5	Schwannoma	Schwannoma	Schwannoma
75	Mujeef	13	M	Suprasellar	Craniopharyngiomas	Craniopharyngiomas	Craniopharyngiomas
76	Jayachitra	30	F	Parietal	Tuberculoma	Tuberculoma	Tuberculoma
77	Gunalan	42	M	Olfactory Groove Anterior	Meningioma	Atypical Meningioma	Meningothelial Meningioma
78	Kaleeswari	25	F	Spinal D12-L2	Medulloblastoma	Medulloblastoma	Medulloblastoma
79	Munusamy	48	M	Frontal	Glioma	Degenerative changes	Oligodendroglioma
80	Valarmathi	20	F	Parietal	Hydatid Cyst	Astrocytoma Grade 2	Pilocytic Astrocytoma
81	Arumugam	28	M	Frontal	Tuberculoma	Astrocytoma Grade 2	Astrocytoma Grade 2

82	Nageshwari	55	F	Parietal	Glioma	Sub Ependymoma	Glioblastoma Multiforme
83	Shamshed Begam	41	F	Para Sagittal	Meningioma	Meningothelial Meningioma	Meningothelial Meningioma
84	Sneeda	21	F	Sellar	Pituitary Adenoma	Pituitary Adenoma	Pituitary Adenoma
85	Rangasamy	70	M	Frontal	Glioblastoma	Glioblastoma Multiforme	Glioblastoma Multiforme
86	Venugopal	50	M	Temporal	Glioma	Fibroblastic Meningioma	Ependymoastrocytoma
87	Deivanai	60	F	Para Sagittal	Meningioma	Meningothelial Meningioma	Meningothelial Meningioma
88	Kanniyammal	26	F	Intra Ventricular	Glioblastoma	Glioblastoma Multiforme	Glioblastoma Multiforme
89	Ganapathy	40	M	Olfactory Groove Anterior	Meningioma	Meningothelial Meningioma	Meningothelial Meningioma
90	Ganapathi	24	M	Frontal	Glioma	Oligodendroglioma	Oligodendroglioma
91	Valliamal	50	F	Frontal	Glioma	Oligodendroglioma	Oligoastrocytoma
92	Venkatraman	25	M	Cerebello Pontine Angle	Schwannoma	Schwannoma	Schwannoma
93	Sridevi	33	F	Parietal	Tuberculoma	Tuberculoma	Tuberculoma
94	Kamini	14	F	Frontal	Tuberculoma	Tuberculoma	Tuberculoma
95	Indumathi	39	F	Parietal	Glioma	Astrocytoma Grade 4	Metastatic Carcinomatous Deposit
96	Karthick	15	M	Spinal C5	Cavernoma	Cavernoma	Cavernoma
97	Abdul Rahman	55	M	Frontal	Glioblastoma	Glioblastoma Multiforme	Glioblastoma Multiforme
98	Rammamma	53	F	Parietal	Glioma	Astrocytoma Grade 2	Astrocytoma Grade 2
99	Jayanthi	35	F	Temporal	Glioma	Schwannoma	Schwannoma
100	Shymala	38	F	Temporal	Tuberculoma	Tuberculoma	Tuberculoma

Annexure I

WHO Classification and Grading

Annexure I

WHO CLASSIFICATION OF TUMORS OF THE NERVOUS SYSTEM

Tumors Of Neuroepithelial tissue

Astrocytic Tumors

Diffuse Astrocytoma
- Fibrillary Astrocytoma
- Protoplasmic Astrocytoma
- Gemistocytic Astrocytoma

Anaplastic Astrocytoma

Glioblastoma
- Giant cell Glioblastoma
- Gliosarcoma

Pilocytic Astrocytoma

Pleomorphic Xanthoastrocytoma

Subependymal giant cell Astrocytoma

Oligodendroglial Tumors

Oligodendroglioma

Anaplastic Oligodendroglioma

Mixed Gliomas

Oligoastrocytoma

Anaplastic Oligoastrocytoma

Ependymal Tumors

Ependymoma
- Cellular
- Papillary
- Clear Cell
- Tanycytic

Anaplastic Ependymoma

Myxopapillary Ependymoma

Subependymoma

Choroid plexus Tumors
Choroid plexus Papilloma
Choroid plexus Carcinoma

Glial Tumors of Uncertain origin
Astroblastoma
Gliomatosis Cerebri
Choroid Glioma of the third ventricle

Neuronal and mixed neuronal – glial Tumors
Gangliocytoma
Dysplastic Gangliocytoma of cerebellum (Lhermitte – Duclos)
Desmoplastic infantile Astrocytoma/ Gangliocytoma
Dysembryoplastic neuroepithelial Tumor
Ganglioma
Anaplastic Ganglioglioma
Central Neurocytoma
Cerebellar Liponeurocytoma
Paraganglioma of the Filum terminale

Neuroblastic Tumors
Olfactory Neuroblastoma (Aesthesioneuroblastoma)
Olfactory Neuroepithelioma
Neuroblastomas of The Adrenal Gland and Sympathetic Nervous System

Pineal Parenchymal Tumors
Pineocytoma
Pineoblastoma
Pineal parenchymal Tumors of Intermediate differentiation

Embryonal Tumors
Medulloepithelioma
Ependymoblastoma
Medulloblastoma
- Desmoplastic Medulloblastoma
- Large Cell Medulloblastoma
- Medullomyoblastoma
- Melanotic Medulloblastoma

Supratentorial primitive neuroectodermal Tumor(PNET)
Neuroblastoma
Ganglioneuroblastoma
Atypical teratoid/ Rhabdoid Tumor

Tumors Of Peripheral Nerves

Schwannoma (Neurilemomas, Neurinoma)
Cellular
Plexiform
Melanotic

Neurofibroma
Plexiform

Perineurinoma
Intraneural perineurinoma
Soft tissue Perineurinoma

Malignant Peripheral Nerve Sheath tumor(MPNST)
Epitheloid
MPNST with divergent mesenchymal and/ or epithelial differentiation
Melanotic
Melanotic psammomatous

Tumors Of The Meninges

Tumors of Meningothelial cells
Meningioma
Meningothelial
Fibrous(fibroblastic)
Transitional(mixed)
Psammomatous
Angiomatous
Microcystic
Secretory
Lymphoplasmacyte – rich
Metaplastic
Clear Cell
Choroid
Atypical
Papillary
Rhabdoid
Anaplastic Meningioma

Mesenchymal, non-meningothelial tumors
Lipoma
Angiolipoma
Hibernoma
Liposarcoma (intracranial)
Solitary Fibrous Tumor
Fibrosarcoma
Malignant fibrous Histiocytoma
Leiomyoma
Leiomyosarcoma
Rhabdomyoma
Rhabdomyosarcoma
Chondroma
Chondrosarcoma
Osteoma
Osteosarcoma
Osteochondroma
Hemangioma
Epitheloid Hemangioendothelioma
Hemangiopericytoma
Angiosarcoma
Kaposi Sarcoma

Primary melanocytic lesions
Diffuse melanocytosis
Melanocytoma
Malignant Melanoma
Meningeal Melanomatosis

Tumors Of Uncertain Histiogenesis
Hemangioblastoma

Lymphomas and Hematopoietic Neoplasms
Malignant lymphomas
Plasmacytoma
Granulocytic Sarcoma

Germ Cell Tumors
Germinoma

Embryonal Carcinoma
Yolk Sac Tumor
Choriocarcinoma
Teratoma
- Mature
- Immature
- Teratoma with Malignant transformation

Mixed Germ Cell Tumors

Tumors of the Sellar Region
Craniopharyngiomas
- Adamantinomatous
- Papillary

Granular Cell Tumor

Metastatic Tumors

World Health Organisation (WHO) Grading System

(Malignancy Scale) of CNS Tumors

Tumor Group	Tumor type	Grade			
		1	2	3	4
Astrocytic tumors	Subependymal giant cell	x			
	Pilocytic	x			
	Low Grade		x		
	Pleomorphic Xanthoastrocytoma		x		
	Anaplastic			x	
	Glioblastoma				x
Oligodendrogliomas	Low grade		x		
	Anaplastic			x	
Oligoastrocytomas	Low Grade		x		
	Anaplastic			x	
Ependymal Tumors	Subependymoma	x			
	Myxopapillary	x			
	Low Grade		x		
	Anaplastic			x	
Choroid plexus tumor	Papilloma	x			
	Carcinoma			x	x
Neuronal/glial tumors	Gangliocytoma	x			
	Ganglioglioma	x	x		
	Desmoplastic infantile ganglioglioma	x			
	Dysembryoplastic neuroepithelial tumor	x			
	Central Neurocytoma	x			
Pineal Tumors	Pineocytoma		x		
	Pineocytomas/Pineoblastoma			x	x
	Pineoblastoma				x

Embryonal tumors	Medulloblastoma				x
	Other primitive Neuroectodermal Tumors				x
	Medulloepithelioma				x
	Neuroblastoma				x
	Ependymoblastoma				x
Central and Spinal Nerve tumors	Schwannoma	x			
	Malignant Peripheral Nerve Sheath Tumors			x	x
Meningeal Tumors	Meningioma	x			
	Atypical Meningioma		x		
	Papillary Meningioma		x	x	
	Hemangiopericytoma		x	x	
	Anaplastic Meningioma			x	

Annexure II

Proforma

Annexure II

Proforma

Study Number:

Name:

Age:

Sex:

Unit:

IP Number:

Clinical Findings:

Investigations:

CT Scan:

MRI:

Surgeons Diagnosis:

Operative Findings:

Crush Diagnosis:

Histopathology Diagnosis:

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Adams J.J Graham D.I and Doyle D. Brain biopsy – The smear technique for neurosurgical biopsies *Biopsy Pathology Series*, Chapman and Hall Pub London, 1981

2. Adekumle M. Adesina Intraoperative Consultation in the Diagnosis of Pediatric Brain *Tumors Archives of Pathology and Laboratory Medicine 2005* Vol 129, No 12pp 1653-1660

3. Annegers J. F. and Paleologos N Intracranial neoplasia in Gorelick P. B and Alter M (eds) *Handbook of Neuroepidemiology New York*: Marcel Dekker 1994, 295 – 313

4. Asha, T . , Shankar, S.K ., Rao, V.T. and Das , S . Role of squash – smear technique for rapid diagnosis of neurosurgical biopsies – A cytomorphological evaluation. *Indian J . Pathol . Microbiol,* 1989, 32 : 3; 152- 160

5. Bailey , P. and Cushing, H. A Classification of the tumors of the Glioma group on a histogenetic basis with a correlated study of prognosis. J B Lippin cott Co, Philadelphia, 1926

6. Barnard, R,O., Logue, v. and Reaves, P.S. An atlas of tumors involving the Central Nervous System London, 1976., 138-139

7. Berkely , B. B., Adams J.H., Doyle, D., Graham, D.I. and Harper, C.G. The smear technique in the diagnosis of Neurosurgical biopsies. *N. Z. Med. J,* 1978, 87: 12 – 15

8. Beuvon F., Varlet P. Fallet- Bianloc, Daumas- Duport C. The "Smear" technique for the extemporaneous examination: diagnostic contribution to neurosurgical pathology 1: *Ann Pathol*. 2000 Oct ; 20(5) : 499-506.

9. Bleggi – Toeres ., L. . F. de Noronha L., Schneider Gugelmin E, Martins Maggio E., Queizoz Telles JE , Martis Collarol Accuracy of the smear technique in the cytological diagnosis of 650 lesions of the Central Nervous System *Diag Cytopath* 2001 April; 24(4) : 293-5

10. Bondy, M. L., Lustbabar ED Buffler, P.A Schull, W.J. Hardy, R.J. and Strong L.C, Genetic epidemiology of childhood brain tumors 1991 8,253 – 67.

11. Burger, P.C., Vogel, F.S., Green S.B. and Strike T.A. Glioblastoma Multiforme and Anaplastic Astrocytoma. Pathological criteria and prognostic implications. *Cancer* 1985, 56 1106/11

12. Bunin, Buckley, Boesel. Risk Factors for Astrocytic Glioma and PNET of the Brain in young children *Cancer* 1994: 3: 197-204

13. Cahill, Hidvegi Crush Preparation of lesions of the Central Nervous System *Acta Cytol*, 1985, 29: 270-285

14. Cappabianca P. Spaziante R. Capriti F Accuracy of the analysis of multiple small fragments of glial tumors obtained by stereotactic biopsy *Acta Cytol*, 1991, 35 : 505-511

15. CBTRUS, Annual report Central Brain Tumor Registry of the United States 1996

16. Chen K T . Crush cytology of Pituicytoma *Diagnostic Cytopath* , Oct 2005 33 (4) : 255-7

17. Cohen A and Modan B, Some epidemiologic aspects of neoplastic diseases in Israeli immigrant population III Brain Tumors, *Cancer* 1968 22 1323-8

18. Colombo. F., Casentinal , L ., Bendetti, A. Biopsy and sterotaxic radiotherapy of cerebral gliomas. *Minerva Med*, 1984, 75: 1327-1331

19. Daneshbod Y., Monabati A.., Kumar PV, Jaghipoor M, Bedayat GR Intraoperative cytologic crush preparation findings in Craniopharyngiomas: a study of 72 cases *Acta Cytol* 2005 Jan Feb : 49(1): 7-10

20. Dastur DK and Lalitha V. Pathological analysis of Space occupying lesions in 1000 cases including children. *J. Neurosurgery* 1968 ; 6 : 575

21. Daumas – Duport , C. Scheithauer. B, Kelly P. Grading of Astrocytomas . a simple and reproducible method. *Cancer* 1988; 62; 2152-2165.

22. Demers P.A., Vaughan, TL and Schommer R.R Occupation, Socioeconomic status and brain tumor mortality: a death certificate based case control study. *Journal of Occupational Medicine* 1991 33, 1001-6.

23. Distefano D. Scucchi LF. Cosenhnot, Bosmanc. Vecchione A. Intraoperative diagnosis of Nervous system lesions. *Acta cytol* 1998 Mar-Apr ; 42(2) 346-56

24. Eisenhardt. L. Cushing H Diagnosis of intracranial tumors by Supravital technique *Am . J. Pathol,* 1930, 6; 541 -552

25. Engelmann, B and Schumacher, U, The Emerging Role Of ABH Blood Group Antigens as Modulators Of Cell Membrane Functions. *Comprehensive Biochemistry and Physiology* 1993 105A, 197 - 203

26. Firlik KS, Martinez AJ, Lunsford LD, Use of cytological preparations for the intraoperative diagnosis of stereotactically obtained brain biopsies- a

19 year experience and survey of Neuropathologists *J. Neurosurgery* 1999 Sep : 91(3) 454-8.

27. Fulling, K.H. and Nelson J.S. Cerebral Astrocytic Neoplasms in the adult: Contribution of histological examination to the assessment of prognosis. *Seminars in Diagnostic Pathology* 1984 152-63

28. Gandolfi A Cytology of a Chromophobe Pituitary adenoma *Acta Cytol*, 1983, 27: 521-524

29. Gandolfi. A , Tedeschi, F. Brizzi. R. The squash smear technique in the diagnosis of spinal cord neurinomas. *Acta Cytol*, 1983, 27: 273-276

30. Gaver RD, MC. GarryP: Delicate longitudinal nuclear grooves in childhood ependymoma *Arch Path Lab Med*, 1994, 118: 919-921

31. Geetha Brain Tumors. A study of 161 cases 1980; 7-24

32. Gonalez Camposa, R. Haynes L.W and Weller. R.O. Scanning Electron Microscopy of Malignant Gliomas. A comparative study of Glioma cells in smear preparations and tissue culture. *Acta Neuropath Berlin*, 1978, 41; 217-221

33. Grays Anatomy, Neurology, Chapter 7, 36th edition 1980

34. Greenfields Neuropathology . Sixth edition David I Graham and Peter L Lansos.Arnold London 1997:2;584-5

35. Green. J.R, Waggenina, JD and Kriegsfeld BA Classification and incidence of neoplasms of the central nervous system in Advances in Neurology Vol15 Neoplasms in the Central nervous System (Eds. Thompson LA and Green J.R.) Raven Press New York, 1976, 51-55

36. Jane J.A and Bektrand, G. A Cytological method for the diagnosis of tumors affecting the Central Nervous System. *J. Neuropath Exp Neurol*, 1962, 21: 400-409

37. Jane J. A and Yashon D . Cytology of tumors affecting the Nervous System Charles c. Thomas sringfield, Illinois, 1969

38. Johnson ES , Nguyen HOP, Nguyen GK Cytology of Central Neurocytoma in intraoperative crush preparation- A case report *Acta Cytol* 1994 Sep – Oct; 38(5); 764-6

39. Kernohan JW, Mabon, Svien A Simplified Classification of Gliomas Proc Mayo Clinic 1949, 24:71-5

40. Kernohan, J.W. and Sayre, G.P Tumors of the Central Nervous System . Atlas of Tumor Pathology section X, fasicle 35 Washington DC Armed Forces Institute Of Pathology 1952

41. Kinoshita , K., fukui m ., KitamuraK., Yonemasu. Y. The smear method for histological diagnosis of the tumors of the Central Nervous System. Shinkei Gekab, 1978, 143-152

42. Kumar P.V, Hosseinzadeh M, Bebayat G.R. Cytological findings of Medulloblastoma in crush smears. *Acta Cytol* 2001 June-August: 45(4):542-6

43. Mahadevan, P., Radhakrishnan v.v., Kartha, C.C and Sandhyaman, S. Histological diagnosis of intracranial space occupying lesions by Squash preparation. Experience wiyh 330 cases. *Abstract from 32nd Annual Conference, IAPM*, 1984

44. Marshall, L.F. Adams J.H. Doyle D., and Graham D.I The histological accuracy of the smear technique for neurosurgical biopsies *J. Neurosurgery*, 1973, 39: 82-88

45. Marshall L.F and Jannet,B; Smear biopsy in neurosurgical diagnosis. *Arch Neurol*, 1973, 29: 124-126

46. Martini, F., De Matter, M., Iaccheri, L, Lazzrin, L, Barbanti – Brodano, G. Tognon, M and Gerosa.M. Human Brain Tumors and Simian Virus 40. *J.of National Cancer Institute* 1995 87, 1331

47. Mc. Menemey. N. H. An appraisal of smear diagnosis in neurosurgery. *Am. J. Clinic path*, 1960, 33:471-479

48. Moarantz R.A. Feigan. I, Ransohoff J.I: Clinical and pathological study of 24 cases of gliosarcoma*, J. Neurosurgery*, 1976, 45:399

49. Nelson,J.S, Tsukada, Y, Schoenfeld, D., Fulling, K., Lamarche,J and Peress,N Necrosis as a Prognostic criterion in malignant supratentorial astrocytic gliomas *Cancer* 52 1983 , 550-4.

50. Nguyen G.K., Johnson Es. Mielke. B.W. Cytology of Meningiomas and Neurilemmomas in crush preparation. A useful adjunct to frozen section diagnosis. *Acta cytol*, 1988, 32; 363-366

51. Nguyen GK Johnson ES, Mielke BW Comparative cytomorphology of Pituitary Adenomas and Oligodendrogliomas in intraoperative crush preparation *Acta Cytol* 1992 sept- Oct ; 36(5): 661-7

52. Pai RR, Kini H, Rao VS, Naik R. Choroid Plexus Papilloma diagnosed by Crush Cytology *Diagnostic Cytopathol* 2001 sep;25(3) : 165-7.

53. Papo, I and Colombo, F. Possibilities and limitations of imprints and smear preparation in the intraoperative diagnosis of endocranial tumors. *Minerva Neurochirugica*, 1959, 3; 134-174

54. Parkin, D.M. Muir, C.S., Whelan S.L., Gao, Y.T. Ferlay, J. and Powel, J, Cancer Incidence in 5 Countries, Volume 6 IARC Scientific Publication 120, Lyon: International Agency for Research on Cancer 1992

55. Patchell RA, Primary CNS lymphoma in the transplant patient. Neuroclinic 1988: 6:297-303

56. Preston Martin S, Henderson B.E. and Yu M.C. Epidemiology of IntraCranial Meningiomas, LA County CA Neuroepidemiology 1983 2, 164-78

57. Preston Martin, S., Mack, W. and Henderson, B.E. Risk Factors For Gliomas And Meningiomas in LA County Cancer Research 49, 1989 6137 – 43

58. Preston Martin, S, Staples, M., Farrugia, H. and Giles, G. Primary Tumors Of The Brain, Cranial Nerves and Cranial Meninges in Victoria, Australia, Patterns Of Incidence And Survival Neuroepidemiology 1993 12, 270 - 9

59. Ramamurthi B, Intracranial Tumors in India. Incidence and variations. *Indn.J.Surg* 1973:58:542

60. Ringertz, Total, Reymond, Grading of Gliomas, *J.Neuropath* 1950, 8:355-80

61. Roessler K, Dietrich W. Kitzv, High Diagnostic Accuracy of Cytological Smears of Central Nervous System Tumors. A 15 year old experience based on 4172 patients. *Acta Cytol*. 2002 Jul. – Aug.: 46(4):667-74

62. Rusell and Rubeinstein, Pathology of Tumors of nervous system 6th edition Chapter I Volume I London Arnold 1998 19 - 30

63. Russell D.S. Krayen Buhl, H. and Cairns, H. The wet film technique in the histological diagnosis of intracranial tumors, a rapid method. *J. Path. Bacteriol* 1937 45:501-505

64. Russell D.S. The wet film technique in Neurosurgery. Recent advances in Clinical Pathology (Duke. S. C. ed) Jand. A Churchill London, 1947 418-425

65. Shanop Shuangshoti, Sumruetai, Kanvisetsri, Intraoperative Diagnostic Brain Smear. Department of Pathology, Faculty of Medicine, Chulalongkom University, Bangkok 1999.

66. Silvermann J.F. Douglas, J.F. Cytopathology of neoplasms of central nervous system. *Acta. Cytol* 1989, 33: 576 – 579

67. Slowinski J. Haratnin. Slowinska, M, Msowka R Smear Technique in the intraoperative brain tumor diagnosis. Its advantages and Limitations, *Neurol Res*. 1999 Jan: 21,(1):121-4

68. Stephen G. Silverberg Principles and Practice of Surgical Pathology and Cytopathology Third Edition Vol3 1997 62 2905 - 3001

69. Sowbhaya, P., Kolluri,V.R.S, Krishna, D.K.SDas, B.S and Narayana Reddy, G N) Intracranial Tumors and Blood Groups *European Journal and Cancer* 1991 21, 221-2.

70. Svien, H.J., Mabon R.F., Kernohan J.N and Adoon, A.W Symposium on a new and simplified concept of Gliomas Mayo Clinic 1949 24, 54-64.

71. Tooth H.H. Some observations on the growth and survival period of intracranial tumors. Brain, 1912 35:61-108

72. Theory and Practice of Histological techniques 5th edition John. D. Bancroft, Chapter 8 Hematoxylin and Eosin pg. 130

73. Turcot, J., Despres JP and St. Pierre, F Malignant tumors of Central Nervous System associated with Familial polyposis of the colon. Report of two cases. Diseases of the colon and Rectum 1959 2, 465-8.

74. Xi Hua Yue, Xin Min Liu Diagnosis of Astrocytoma in crush preparation. *Acta cytol* 1987, 31:83-84

75. Zulch K. J Histological typing of tumors of the Central Nervous System. International Histological Classification of tumors 21 , Geneva: WHO, 1979